

## Let's hear it for the PhD

EIGHTEEN months ago the House of Commons Expenditure Committee issued a report on Postgraduate Education (House of Commons Paper No. 96). The general thrust of the document was that growth in postgraduate education had been insufficiently controlled, that postgraduate education should be shaped less by student demand and more by the needs of the economy and society as a whole, and that there should be a major change of emphasis towards postgraduate education being for those who had already experienced the world outside university. We thought at the time that it was a poor report as far as it applied to the PhD, and now that the House of Commons has just got round to debating it, we still think it's a poor report.

Much play was made, during the short debate, of the lack of any formal response by the Department of Education and Science to the document. By way of explanation the Under-Secretary (Miss Joan Lestor) pointed out, rather apologetically, that a large number of bodies were interested and it took time to collect and consider their views. Since the report is a potential threat to the whole way of doing academic research and since the bodies with an interest all have their offices within a bus-ride of the department one wonders why the delay in standing up for the PhD—could it be that the department is in broad agreement with the report? Miss Lestor's opaque speech gave no clues.

Mercifully, at the very end of the debate, one member, Dr Jeremy Bray, was prepared to stand up and plead for more understanding of the basic purposes of postgraduate education. "We need a permeation of the attack on the problems of modern society by the robustness, objectivity, and the exposure to the test of experience, which is the characteristic of good research. Any activity which fosters that and which seeks to spread those qualities within our society should not be lightly brushed aside." An unashamedly Popperian viewpoint probably lost on the House which immediately went on to discuss police recruitment.

The PhD degree is a relatively easy target to attack because its recipients do not seem to confer on society the same sort of benefits as, say, medical doctors. More recycle into the educational system than go elsewhere (not that industry, commerce and the Civil Service wants them all that much). Many get their degree and then

seem to throw it all away by going on to do something apparently totally unrelated—an offence which is widely practised, indeed encouraged, after 16 years of education but totally unacceptable after 19. And they are alleged to be strangely unresponsive to society's needs, although they are invariably supervised and guided by more senior people.

What ought to be more widely known is that at least as far as the research student is concerned the transition from undergraduate to postgraduate work is such a change in style that few of those who drift into research for fear (as Mr Neil Marten put it) of the outside world survive long. To switch from a situation in which every problem has an answer (and preferably one that can be written down in a couple of sheets of examination paper) to a situation in which one is not even sure that the problem is worth asking, let alone whether it is answerable, is to expose oneself to a new and often icy world. And if it is done at all, it must be done young. The record of those who do come back for a research degree after several years in, say, industry does not make a compelling case for the post experience grant.

Many take the PhD degree but do not then seem to capitalise on it; this is easily seen as waste, and no doubt the Expenditure Committee could see it as that, but substantial numbers of students realistically face the fact that after three years of conducting research they are not cut out for a lifetime of it. In those three years, though, they will have learnt a whole new philosophy and methodology (and learnt by experience, not instruction). There are other places where such experience is needed than in the laboratory.

Finally, what should shape postgraduate education—the needs of the economy and society or student demand? The committee leaned to the former and there is much to be said for manpower planning in higher education. But society is generally only capable of servicing its present needs, and somewhere there has to be some very careful thinking about what the community will need thirty years hence. The future of a technological society is at least partly in the hands of the emerging student; to deny him the right to choose what sort of society he wants to work for is to try and prevent the further evolution of society—however reasonable the arguments on the other side may seem. □



# Seeds of hope

The improved varieties of staple crops which hold out the eventual hope of feeding the world's growing population, will depend for some of their most valuable characteristics on the genetic raw material contained in the old indigenous varieties of crops cultivated by peasant farmers for thousands of years. But this pool of inherited variation is rapidly drying up because of the success of the very varieties it helped to create. The primitive 'landraces' and their wild relatives have in the past proved an invaluable source of resistance (worth many millions of pounds) to serious pests and diseases, and have provided genes for many other desirable characters such as improved nutritional quality and adaptations to drought or cold. The serious implications of the rapid disappearance of this reservoir of valuable genes is now recognised in many quarters, and work to conserve the variation which remains is under way throughout the world. Eleanor Lawrence reports on one of the first institutes specifically set up to tackle this problem as it enters its second decade.

THE Genetic Resources Unit established by the FAO within the Aegean Regional Agricultural Research Institute (ARARI) just outside Izmir, on Turkey's Aegean coast, started life in 1964 as an experiment unique at the time. It was the first such unit specifically set up to deal with the collection, storage and evaluation of the rapidly vanishing genetic variation in cultivated crops within a region holding a large part of the remaining stocks of primitive cultivars and wild relatives of some of the most important temperate crops—wheat, barley and grain and forage legumes.

The concept of a 'gene bank' itself was hardly new even in the early 1960s. Plant breeders had collected useful material from all over the world to

incorporate into plant breeding trials, but material not of immediate use had often been discarded. Large reference collections of seed of important crops were held at the National Seed Storage Laboratory at Fort Collins in Colorado, and in the USSR, where the now irreplaceable collections made by N. I. Vavilov and his coworkers in the 1920s and '30s were kept in Leningrad and at other centres throughout the country. Collections of various types and sizes were also held throughout the world.

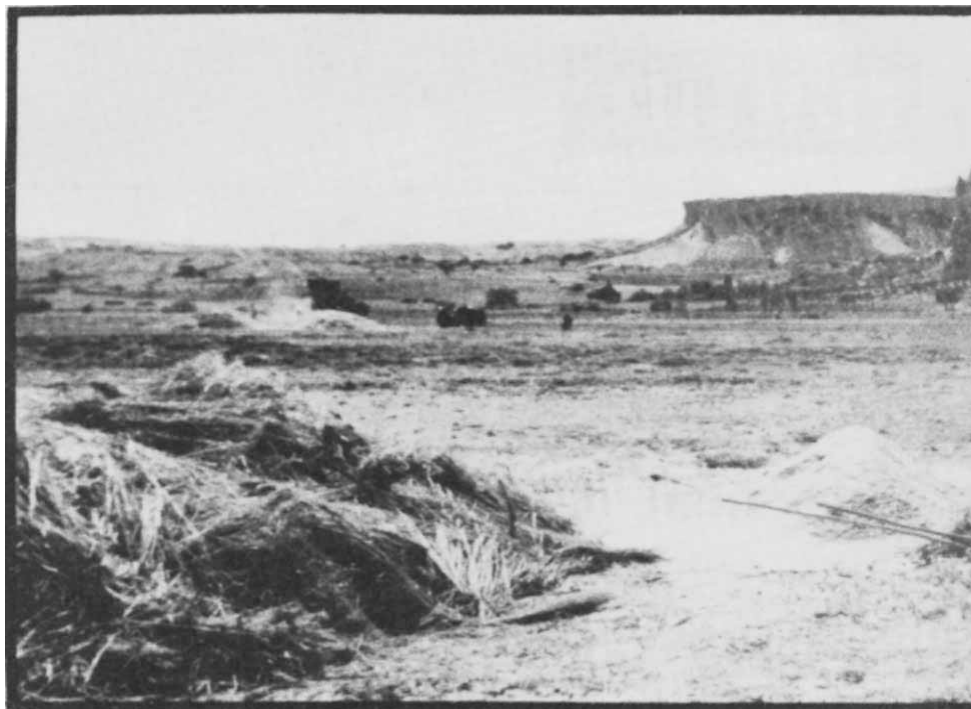
But there were as yet no medium-sized institutes, actually located in the countries where the greatest genetic diversity occurred, which had as their primary aim the systematic collection, and on-the-spot conservation and study, of valuable seed material, with the additional aim of screening and describing the material and making it freely accessible.

The importance of the Near East (stretching from Turkey to Pakistan in the east and Lebanon in the south) as a centre of diversity for many temperate crops stems from its complex geography and its long continuous history of agriculture. The Near East is the cradle of modern 'western' agriculture, based on wheat and barley as staples, which were first cultivated there some 10,000 years ago. Archaeological evidence shows that a mixed agriculture based on wheat and barley had developed throughout the region by the 8th century BC. Barley, emmer wheat (tetraploid *Triticum dicoccum*, a relative of the modern *durum* wheats), the modern hexaploid bread wheats (*T. aestivum*) and the diploid einkorn wheat (*T. monococcum*), still cultivated in parts of Turkey today, have all been found at archaeological sites dated be-

tween 6000 and 7000 BC.

But today, according to a survey carried out by FAO and the International Biological Programme published in 1973, improved varieties of both *durum* and *aestivum* wheat have very nearly replaced local cultivars except for remnants in the more remote regions. The wild progenitors of both wheat and barley can still be found and are even increasing in localities where they happen to be within protected 'national park' areas. Today wild barley flourishes in the ruins of Ephesus.

If this gene pool of inherited variation built up over thousands of years of evolution, selection and diversification into a wide range of habitats, is allowed to disappear completely, the plant breeder's ability to respond to a changing world situation will be severely restricted. These traditional 'landraces' and their wild relatives are often the only source of resistance genes to a wide variety of serious pests and diseases, such as stem rust in wheat. The old landraces are sometimes not very productive, and some do not respond to fertiliser treatment, but locally adapted populations can withstand conditions of drought or cold for instance which would soon lay low most modern varieties. These characteristics are needed to incorporate into breeding programmes with the generally more productive, modern varieties. Also, with fertilisers and pesticides growing more expensive each year, modern varieties which depend for their high yield on generous inputs of fertilisers may become less attractive. Traditional cultivars which produce good yields under less favourable conditions may prove a more rational basis for future breeding programmes







Harvest in Turkey: reservoir of genetic resources

for some areas.

In the decades since the Second World War, Turkey has seen widespread changes in agricultural practice. Changes in land use, introduction of improved varieties and modern methods of agriculture including the use of herbicides for weed clearance, have hastened the disappearance of the old locally-adapted populations.

In 1961, the Turkish government asked for international aid to research into problems posed by the changeover to modern systems of agriculture. In 1963 the United Nations Special Fund (now incorporated into the UN Development Programme) allocated funds and with FAO as the executive agency the 'Izmir Project' began. The Turkish request for aid coincided with FAO's own wish to establish a centre for the collection, study and distribution of forage legumes and grasses, and also with a growing consciousness of the urgent need to survey and collect rapidly disappearing germplasm of important crops. Over the nine years during which the project was financed by UNSF/UNDP, the Turkish government provided a total of \$980,000 worth of land and staff salaries plus \$110,000 in cash, and the UN around \$1,290,000.

The original aim was to establish a Crop Research and Plant Introduction Centre, which as well as carrying out genetic resource surveys and conservation would maintain and initiate breeding programmes and also serve as the point of introduction for new varieties entering the country from abroad. The introduction of plant material from another country is always a risky operation because of the possibility of disease being brought in accidentally. Quarantine procedures for screening incoming

material and certifying outgoing seed as disease free are an important part of the work of any centre involved with the distribution of plant material. It was also essential for a unit concerned with genetic resource conservation to keep a finger on the pulse of plant introduction so that they could foresee when the introduction of new varieties into previously undeveloped areas might threaten valuable sources of primitive cultivars.

But the early days of the project were dogged by organisational problems. At the time of its establishment, the Turkish government was also in the process of setting up its own agricultural research institute on the same site using the same funds and facilities provided for the FAO/UNSF programme. It proved logistically impossible to keep the two elements separate, and the original interpretation of the project by FAO as being one primarily of building an institute with an essentially international outlook for the collection, conservation and use of crop genetic resources became to some extent engulfed in the Turkish aim of building an institute of much wider and more immediate value to Turkish agriculture. This was recognised in a merger with the Ministry of Agriculture institute, the present ARARI, in 1967 and the present status of the genetic resources unit within ARARI. From 1970 until UNDP ended its financial aid in 1973, all UNDP/FAO funds were concentrated on the development of this discrete unit within the institute.

The original programme also proved to be overambitious given the limited financial resources and organisational difficulties. The present efficient and streamlined genetic resources unit has,

for the time being, had to discard its original plans for full evaluation of the material collected and now concentrates on collection, storage, and computer documentation of the extensive herb-arium and seed collections. Since 1968, there has been a systematic collecting programme in crops particularly endangered by the changing patterns of agriculture. More than 2,600 samples of cereals, 3,759 samples of grain and forage legumes, 615 samples of vegetable and spice crops and 1,513 samples of 'industrial' crops (including over a thousand samples of opium poppy seeds) have been collected from all over Turkey; additional accessions have been received from other countries, and deposited in the excellent cold storage facilities completed in 1968.

The poppy seed collections were made in 1972, under the impending ban on opium poppy cultivation. No collections had been made previously so when the government ban was announced an emergency collection of seed stocks was mounted. Although FAO does not condone the traffic in opium, *Papaver somniferum* and *P. bracteum* are also important oil seed crops in more than 2,000 villages in Central Anatolia and it was hoped that varieties with high oil content and low morphine content could be selected for restocking. With the full cooperation of the government, local agricultural extension staff were quickly trained in the basic techniques of collecting and over 1,000 samples were gathered in the one season before cultivation stopped.

This exercise also proved an excellent example of the greater efficiency of collections organised and carried out by trained local staff, familiar with the terrain, language and customs of the country. The one-off expeditions mounted by foreign teams, unfamiliar with country and language can sometimes prove unproductive, and one of the roles of the Izmir centre has been to provide support and advice for the many expeditions which visit Turkey, attracted by the diversity of its natural and cultivated flora.

A survey and collection of the wild and traditional varieties of fruit and nut trees, such as almond, walnut, apple and peach, which were first cultivated in the Near East and grow wild in Turkey, has also been made. Although these are not staple crops, they are particularly vulnerable to changing land usage. The traditional mixed orchards where the old cultivars are grown are not being replaced, and large commercial plantations of new varieties are being planted instead. The wild species are also disappearing rapidly. Unlike herbaceous plants, tree germplasm is often difficult to conserve



as seed. A living collection assembled at Izmir was finally abandoned when it proved too difficult to maintain and evaluate the living trees in conditions far removed from those to which they were adapted, and attention was instead concentrated on selecting the best lines as a basis for varietal improvement. *In situ* preservation in protected areas probably gives the best chance of preserving the wild varieties.

The ending of UNDP aid in 1973 marked another change in emphasis for the Izmir centre. Since 1970 the international contributions had gone solely to the genetic resource unit, and this now continues with funds from the Swedish International Development Authority through FAO, which have been promised until mid-1976. At the same time, the scope of the genetic resource project was formally widened to take in the neighbouring countries of Syria, Iraq, Iran, Afghanistan and Pakistan, under the title of the Near East Project, which has now been in progress for one year, and aims, with Izmir as a focal point, to coordinate the national efforts in these countries, all important areas of genetic diversity.

The future role of Izmir as the main regional centre is now in some doubt, however, despite its excellent technical facilities, scientific record and core of highly trained, experienced Turkish staff. The problems arise from its present position within a government-controlled institute, where although funded internationally, it is still under the ultimate control of the director of the ARARI, and therefore not completely independent.

Financial support for the Near East Project after mid-1976 is to be channelled through the newly-established International Board for Plant Genetic Resources (IBPGR) which is considering an alternative for the regional centre to serve this programme. In the present reappraisal of world-wide genetic resource conservation at present being carried out by the IBPGR with a view to linking existing programmes into a global network, it would prefer where possible that the regional centres be autonomous and be able to take a truly international view of their essential function of making the seeds they collect and store freely available. The Consultative Group on International Agricultural Research, the parent body of the IBPGR, is setting up a new international agricultural research centre to serve the Near and Middle East in the Lebanon and this might prove a satisfactory location for the regional centre for genetic resource conservation as well, with Izmir continuing as a national centre—perhaps under international funding if necessary.

The Izmir project was a milestone in

### Distribution of seed samples

|      | Turkey | Abroad | Total |
|------|--------|--------|-------|
| 1967 | 2,975  | 160    | 3,230 |
| 1968 | 214    | 2,624  | 3,214 |
| 1969 | 1,473  | 265    | 3,214 |
| 1970 | 3,668  | 195    | 1,738 |
| 1971 | 3,474  | 1,672  | 5,146 |
| 1972 | 2,186  | 976    | 3,062 |
| 1973 | 447    | 1,578  | 2,025 |

Over the years samples of about 75% of the collections have been sent overseas to scientists and plant breeders on request, with the greatest demand being for wheat, barley and grain legumes. Complete duplicate collections of the cereals have been sent to Canada, chickpeas to India, Holland and the Lebanon, lentils to Argentina and the Lebanon and broad beans to Sweden, East Germany, the United States and Australia. When seeds are sent they are always accompanied by requests that any information on performance and characteristics be returned so that records can be kept up to date.

the transition of genetic resource conservation from a state of individual effort into an internationally coordinated effort, but like many pioneer projects is now hampered by limitations imposed by its origin at a time when the problems of organising genetic resource work on such a scale were imperfectly appreciated.

Another candidate which has been put forward as an alternative regional centre is Ege University near Izmir.

There would be many advantages in locating a centre at Ege, which already has a good department of agriculture, existing collections and facilities including cold storage. Future work on genetic resources, once the initial urgent need for collection has been satisfied, can be usefully diversified into basic research in plant genetics, taxonomy, pathology and ecology which will illuminate the potential of the collected material for use in plant breeding programmes. The present FAO Project Officer feels that two projects especially would be particularly appropriate. As legumes always grow naturally in a symbiotic association with nitrogen-fixing rhizobia, which are specific for different species and probably as genetically variable as their plant hosts, a gene bank of rhizobia complementary to the legumes seems essential for the conservation of the natural symbiotic system, and would also prove invaluable for bacterial geneticists who are now trying to breed more efficient strains of rhizobia and attempting to extend their host range. The UN Environment Programme is considering plans at present for the international coordination of *Rhizobium* collections.

Such a centre would also be an ideal base for work in the variation found

between different races of plant pathogenic fungi, which poses a problem for the breeder trying to incorporate resistance into the plant, and for surveys of the extent of disease resistance within areas where it has arisen naturally.

A completely new regional genetic resource centre, wherever it is eventually located, will, however, take some time to put into commission and time is in short supply as far as exploration and collection are concerned. The facilities at Izmir will certainly be needed more than ever in the next year or so. Most of the Near East countries have vigorous programmes of agricultural improvement, which within the next decade will further reduce the areas where the older varieties still remain.

Other changes in the Near East Project may be envisaged under future IBPGR funding. The anomalous position of the oil producers Iran and Iraq as recipients of international aid is unlikely to continue. Although the international organisations will be happy to provide expert help and training facilities for young scientists, it is hoped that Iran and Iraq can be persuaded to become contributors along with the other donor countries which comprise mainly the industrialised nations. Although scientists within these countries are fully aware of the necessity to conserve their remaining sources of variation in cultivated crops, it is sometimes difficult to persuade governments that such essentially long term projects should be supported. But the Izmir Project showed that for a comparatively modest sum, compared with the thousands of millions of dollars often spent on prestige technology, irreplaceable sources of variation can be safely banked for the future. □

# international news



THE Apollo-Soyuz link-up has undoubtedly proved a salutary exercise in the logistics and diplomacy of international scientific cooperation, and as such has merited not only congratulatory speeches from Messrs Ford and Brezhnev, but also a special commemorative perfume EPAS (Experimental Project Apollo Soyuz, bottles by Revlon, contents by Novaya Zarya) distilled in honour of the occasion. But while the politicians, planners, translators, coordinators and language instructors congratulate themselves on a well-earned triumph, the question remains: what precisely did the joint mission achieve in the scientific field which could not have been accomplished by individual national missions?

Of the "international" experiments carried out by the mission, the artificial solar eclipse made by undocking the two craft, and manoeuvring them into a position in which the Sun was eclipsed by Apollo, and the corona photographed from Soyuz could, theoretically have been effected by two spacecraft of the same "nationality".

The unilateral experiments, *a fortiori*, are likewise independent of the joint nature of the mission. Their juxtaposition in a joint mission does, however, stress a slightly more exotic trend from the American side, since, in

## Sweet smell of success

from Vera Rich

contrast to the fairly routine astrophysical, geographical and biological experiments of the Russians (photography of the zodiacal light, investigation of refraction and transparency of the upper atmosphere, the study of the effect of weightlessness and other conditions of spaceflight on fish embryos and micro-organisms), the American programme included the growing of crystals, the production of lithium fluoride fibres, the casting of magnetic materials, and two electrophoresis experiments (one of West German origin) dealing with the separation of live cells and the isolation of enzymes. It must be remembered, of course, that this Soyuz flight is, from the Soviet point of view, simply a rather more spectacular member of a continuing series, whereas although NASA has two Apollo craft remaining in care and maintenance, there seems no likelihood that with current budgetary restrictions it will be able to launch them.

This, to a certain extent, vitiates the only practical purpose of the joint

mission. In a television interview, a high-ranking Soviet spokesman for the "Interkosmos" council for co-operation in space research stated that according to the International Space Treaty, the contracting parties are bound to implement all possible means to render mutual aid and assistance in the case of emergency. International ventures on perfecting means of docking and rendezvous will therefore continue. Moreover, he said, the future of space research lies in international space stations. The possibility of a Soviet space-craft coming to the aid of a U.S. craft in distress is clearly a tempting prospect. The helplessness of the Soviet cosmonauts to effect a salvage operation in the case of the Apollo-13 near-disaster caused keen heart-searching among those who realized what a propaganda boost such a rescue would have been. In all events, unless the U.S. Congress will release the necessary funds, it seems unlikely that Soviet spacecraft will be able to practice the necessary rendezvous and docking manoeuvres, except on each other.

In an ideal future, without budgetary restrictions, where international teams can work together in manned space stations, or even interplanetary flights, one outcome of the current





Apollo Soyuz mission will have to be borne in mind—the increased physical stresses observed from the telemetered medical data. The crews were, of course, old for the task, and Slayton had a history of cardiac trouble, but age does not seem the only explanation. A number of factors may well be involved—the intense political significance with which the mission was loaded and the almost embarrassingly detailed TV coverage. But these are factors which would with time, gradually fade as international flights became commonplace. It is possible, however, that the strain of operating in a bilingual situation, added to all the other in-flight stresses, may have

proved more wearing than expected. Perhaps therefore the most profitable follow-up of Apollo-Soyuz would be a detailed study of a multinational crew in a simulated long-term mission, with a similar but uninational crew as control experiment, in order to study in depth the additional stresses, if any, caused by international cooperation.

*John Gribbin adds:* If Apollo-Soyuz was anything more than a political stunt, it was an attempt to pave the way for future joint missions in space. The most glamorous manned mission of the foreseeable future (by the turn of the century) would be a visit to Mars, where the great cost would surely require a global, rather than any national, effort. But before then, and perhaps within five or six years, there is plenty of scope for significant co-operation in Earth orbit.

Curiously, the plans of Soviet and American space scientists dovetail together so well that, politics apart, they seem made for each other. In the best traditions of Tsiolkovski, the USSR is working towards a reasonably large permanent (or semi-permanent) manned space station—but they seem to be intending to build it with the aid

of what are now conventional rockets. On the other hand, the present US effort is towards a superb haulage vehicle for Earth orbit trips, the Shuttle. What could be more natural than to use the Shuttle as a means for supplying the space station of the 1980s?

NASA at least is already well aware of the needs for international collaboration on future projects, and perhaps the most significant single item to be carried by the Shuttle, the Space Laboratory, is being designed and built in Europe, as the European Space Agency's contribution to manned spaceflight. In the heady excitement of last Thursday's golden handshake, the dream of collaboration involving the US, USSR and Europe to fulfill science fiction predictions of permanent orbiting Earth stations seemed almost to be a reality; in the more down to Earth days to come, it seems more likely that any such dreams will be blighted by the attitude (on both sides) typified by a remark attributed to one of the US astronauts to the effect that "I like the Russians we've worked with. But that doesn't mean I have to like their lousy system of government." □

A cooperative research programme between the USA and Egypt in the area of remote sensing applications to a national resources survey (in geology, agriculture groundwater and so on) was initiated in 1972 by a proposal submitted by Dr M. Abdel-Hady (an Egyptian scientist who is now Professor at Oklahoma State University, OSU), to the National Science Foundation (NSF) and the Egyptian Academy of Scientific Research.

The project was approved and funded by Oklahoma State University and the NSF for a three-year period ending in 1975, and Professor Abdel-Hady was granted research leave from OSU to direct the effort from Egypt.

The programme so far has covered work in the following areas:

- A geological, structural, drainage and mineral resources survey for the northern region of the Aswan Dam reservoir basin, comprising an area of 68,000 km<sup>2</sup> on both sides of the river.

- Infrared thermal imaging from aircraft over an extensive area south-west of Cairo. From this investigation, in a typical arid climate, lithological anomalies were significant in demonstrating the role of remote sensing techniques in the discovery of important economic minerals.

- Several studies of the use of remote sensing techniques for the survey and early detection of fungus and nematode diseases in regions of Egypt where important economic crops are grown.

- Geological, water resources, potential oil, mineral resources, and structural maps of the Sinai Peninsula from ERTS-1 Satellite images (under contract with the Ministry of Reconstruction and Housing in Egypt).

- Thermal (infrared), magnetic, radiometric and multispectral photographic aircraft surveys over the entire Suez

## Remote sensing in Egypt

*from Salah Galal, Cairo*

Canal Zone, to provide basic surface and subsurface geological maps for a strip 20 km wide, along the entire length of the canal to assist the redevelopment and reconstruction projects in this area and to provide data about the subsurface to the international construction companies in charge of designing the proposed tunnels under the canal.

Several other investigations dealing with agriculture, land reclamation, mineral resources, groundwater and the environment are now proceeding, and more than 10 technical reports and research papers are being prepared from the results of these investigations. These will include studies of the Suez Canal zone (surface and subsurface geological and groundwater survey); the north-west coastal region of Egypt (geological and soil survey, groundwater survey, soil salinity, and agricultural

survey); and the Salheia Project (10,000 km<sup>2</sup> between the Nile Delta and the Suez Canal). In this project a regional and detailed geological, soil hydrological and crop survey is being carried out, with the aim of reclaiming 10,000 acres of land.

Other projects now being negotiated include a crop survey inventory and early detection of some nematode and fungus diseases in major economic crops in Egypt; a survey of iron ore deposits over large areas of the Western Desert; a regional geological soil and water resources survey for 100,000 km<sup>2</sup> west of Aswan; and a regional geological, structural and petroleum and mineral resources survey of a large area in the Western and Eastern Deserts of Egypt.

Regional groundwater investigations and mapping of groundwater reservoirs are planned for the Nubian Sandstone in Egypt, northern Sudan and Libya, and regional and general area reconnaissance will take place in the El-Sudd region in Sudan for the planning and location of the Jongoli Canalisation Project (an important water resources and water conservation project planned jointly by the governments of Egypt and Sudan). Cooperation in carrying out this investigation by satellite image interpretation and by means of aircraft is now being negotiated with representatives of Egypt and Sudan at the Higher Commission for the Upper Nile Waters, Ministry of Irrigation.



## Brazil and Germany do a nuclear deal

from Ian Bridges, Bonn

IN Bonn at the end of June foreign ministers Hans-Dietrich Genscher of the Federal German Republic and Antonio Azeredo da Silveira put their signatures to an epoch-making treaty. Under its terms the two countries are to develop, using German know-how and Brazilian capital and uranium, a complete nuclear fuel cycle, from mining to waste disposal. This was the German's first breakthrough in the complex game of world-scale technological and commercial diplomacy. The range of reactions to the deal is a reflection of the many and diverse planes on which it will have an impact. Within Germany, the accent was put on the value of the commercial contracts and their spin-off in balance of payments terms. In Brazil, relief at ensuring energy supplies for the future, when hydroelectric power will have been stretched to its limits, mingles with satisfaction at an assertion of independence from Uncle Sam. In the United States, Congress echoed to expressions of high moral indignation that Germany, of all countries, should have given the Brazilians the wherewithal to "make the bomb".

Shrewder observers noted that the deal had long-term political and strategic implications, relieving the Federal Republic of dependence on US enriched uranium in the closing decades of the century. And in the background, ecologists lamented that it opened the way to a further spread of nuclear power, with potentially disastrous implications for the future of mankind, whilst scientists speculated about this as a test case for the effectiveness of control over the peaceful use of a new enrichment method.

Technically, the nuclear complex that is to be built at Angra dos Rios, on the Itaorna Bay 130 miles of Rio de Janeiro, represents a double first. It is the first complete nuclear power cycle to have been sold. This indeed was the key selling point with the Brazilian government, who could not obtain a similar offer from Westinghouse, whose single 600 MW standard plant is currently being built within a few hundred yards of the site for the new complex.

By the time the cycle is completed (within 10 years), it will cover prospecting for and mining of uranium within Brazil, which is known to have such resources; a uranium enrichment plant; two completed 1,300 MW pressurised water reactors, with another six reactors on the way; a pilot plant for making reactor fuel elements, with a commercial plant to follow; and a

plant for processing used fuel. But in this list, one thing stands out—the enrichment plant. For this is the first commercial sale of the method known as nozzle or vertical wall centrifuge (German: *Trenndusen*). This has been developed by the Federal German nuclear research centre in Karlsruhe—and in parallel by the South Africans—but has not been used commercially so far.

It was mainly the ability to sell an enrichment process—plus their proven engineering ability across the whole field—which gave the Germans the edge over the Americans. Brazil's economic planners had begun to find themselves doubly squeezed on the energy front. With maximum exploitation of the country's considerable hydroelectric power resources already foreseeable, they had made the switch to oil when the world oil crisis hit them. For a country which had just begun to master about the worst runaway inflation rate in the world, this was a shock. But thinking in terms of nuclear power as a medium-term alternative, they ran into the hard fact of the predicted enriched uranium shortage, already making itself felt, and due to be worse at the end of the 1980s. So the nuclear choice looked like restoring a dependence on the US which they were trying to break.

The answer was to develop a full fuel cycle enriching their own uranium. But the gaseous diffusion method, the only one the US companies could theoretically have provided, could not be made available on non-proliferation grounds, because it can be used to enrich up to the levels needed for military uses. The Anglo-German-Dutch centrifuge method is also subject to the tightest restrictions, under the secret clauses of a deal with the United States, and is in any case not yet available commercially. The German method, on which the scientists in Karlsruhe have concentrated, is generally recognised as being "proliferation safe"—meaning that it can only effectively be used to enrich up to the low levels, 3.5% or so, needed for peaceful uses. It seems that any adaptation of the vast installations to achieve higher levels, would be so easily controlled.

costly and so conspicuous as to be In these circumstances, the wave of protest in the US Congress against the German-Brazilian deal, specifically on the grounds that it meant "giving the Brazilians the bomb", seems hard to explain, unless resulting from inadequate technical knowledge. Although Brazil is not a signatory of the non-proliferation treaty, the Germans made it a condition of the sale that Brazil should accept controls by the International Atomic Energy Agency

in Vienna. The powerful pressure which the US government also tried to put on the Federal Republic, against the deal, seemed to be more general. But as the Germans tended to point out, if the danger foreseen was that plutonium from the reactors, after their first period of operation, was seen as giving Brazil a military potential, this is a danger equally present in US sales of reactors to countries at least as potentially dangerous as Brazil, such as Iran or Egypt.

One expert has suggested a third possibility: one of the inventors of the centrifuge spent many years after the War, when working on the project, in Brazil before returning to Germany, and it would thus be surprising, said this source, if the Brazilians had not discreetly gone on with the centrifuge; but this was queried by others who pointed out that the bottleneck in centrifuge development was not the invention itself but the difficult high-temperature technology of the bearings, something the Brazilians are not equipped to master. In short, if Brazil is suspect as a potential maker of bombs, the breakthrough is going to come anyway. As for innuendoes that the deal also gives the Germans the potential of developing a bomb together with the Brazilians, there seems no foundation for them—at least as long as the Federal Republic retains its present political balance.

From the Federal German point of view, in any case, there is no doubt that the Brazilian deal was pushed through essentially for its commercial value. For the Germans it was a lucky break when they were beginning to despair of ending the Americans' domination of the world market for nuclear technology and engineering. An estimated 15,000 million DM of public money has been poured into the nuclear industry, which is now geared up to produce and sell reactors commercially. Kraftwerk Union AG, joint subsidiary of Siemens and AEG (though AEG are currently selling out) can produce and sell six reactors a year. Given a six-year construction cycle, this means a potential of 36 contracts running in parallel. Currently, there are orders for only 17. Except in neighbouring countries—Austria, Switzerland, Holland—KWU has almost lost out to Westinghouse or General Electric—not least because of the all-out diplomatic back-up enjoyed by the Americans. Thus the Germans recently won an order from the Shah of Persia for two reactors—only to see the Americans carry off a contract shortly afterwards for a programme of eight over a 10-year period.

In Brazil, precisely the reverse has happened. The starting-point was a straight bi-lateral inter-governmental

pact, signed in 1969, on the exchange of scientific information. From the start the Brazilians showed the most interest in the nuclear sector, and soon talked about a full fuel cycle, but were not taken seriously. In 1972 the Germans made a bid for the reactors the Brazilians had decided on, but when they saw Westinghouse carry off the first contract they almost gave up. Then, in the spring of 1974, came a request for the full fuel cycle, this time serious. The talks, on which German industry was brought in, were kept secret for a long time.

When it did come out, in the early summer, the news was an enormous fillip for a German economy reeling under bad news. For years, the prosperity of the Federal Republic had been export-led, with booming sales of investment goods abroad balancing out the staid performance of private consumption within the country. In 1975 exports had begun to flag, reflecting the recessions elsewhere in the industrialised world. Of the investment in the nuclear programme planned with the Brazilians, an estimated 12,000 million DM was to be spent in the Federal Republic over 15 years. There was guaranteed work for the 13,000 strong skilled workforce at KWU, plus sub-contracting to 300 or more firms for 80% of the material to be used. But there can be no doubt that nuclear development proper will spearhead a wider commercial breakthrough. There will clearly be a need for steel produced on the spot, and for a range of engineering firms; and around this tremendous source of electric power, fertiliser and chemical plant will proliferate.

Far more than was generally realised at first, the Federal Republic and Brazil have gone into partnership in the business not just of producing nuclear power, but of supplying the kits for others to do the same. The briefest summary of the full range of cooperation arrangements illustrates this.

- a joint company has been set up to carry out prospecting and mining of uranium. A Brazilian state company, Nuclebras, has 51% and Germany's Uran Gesellschaft the other 49%.
- the same Nuclebras will take part in developing the vertical wall enrichment plant, which will be built jointly in Brazil by Steag and Interatom.
- KWU and the specialised company RBU are to build first a pilot plant and then a commercial one for making nuclear fuel elements for Brazilian reactors; and significantly Nuclebras will have 70% and KWU 30% in a separate company set up to market fuel elements.
- for re-treatment of nuclear fuel, Nuclebras will have two German firms

THAT much-publicised proposal to reestablish a science policy office in the White House, announced by President Ford in May, is making glacial progress through Congress, and it now seems certain that the new arrangement will not be in place until late autumn. That would be too late for the office to play much part in deliberations on the 1977 budget and, with the Presidential elections by then in full swing, it may be difficult to persuade a top flight person to head the office for what could be a short stint.

The House Committee on Science and Technology, which is dealing with the proposal in the House, completed hearings on the measure last month, and it is not likely to take any formal action until after Congress returns from its August recess. In the Senate, meanwhile, the proposal has been referred to three different committees, and joint hearings are being planned for September. Congressional staff members concerned with the legislation suggest that it will be at least October before a bill is passed and sent to President Ford.

Ford's proposal, which was formally spelled out in a bill sent to Congress early in June, would establish a small office consisting of about 15 people—a number which was "pulled out of the air", according to testimony given by Vice-president Rockefeller—and a budget of about \$1-1.5 million a year. Although it would be concerned chiefly with helping to formulate Administration policy on non-military science and technology, the office would play a rôle in military matters "when asked".

Congress is likely to give Ford

pretty much what he asked for, although it is likely to stipulate that the head of the office, who will bear the title of Science Adviser to the President, should be approved by the Senate before he is officially appointed. It is also possible that Congress will spell out the office's areas of responsibility in some detail, particularly in regard to its rôle in military science policy making.

During the House Science and Technology Committee's hearings, the proposal was warmly endorsed by several prominent scientists who had long been protesting former President Nixon's decision, in January, 1973, to scrap the White House science policy arrangements established by President Eisenhower. Having persuaded President Ford to reverse his predecessor's decision, however, the proposal's supporters have won only half the battle—the other half is to ensure that the arrangement is used effectively when it is in place, and that may be more difficult.

● Echoing warnings issued in a recent report of a committee of the National Academy of Sciences, the Geological Survey, which is part of the Department of Interior, pointed out last week that the United States is becoming increasingly dependent on imports of several important minerals. The survey urged that a national policy aimed at conserving scarce minerals and substituting others in some uses, should be adopted. By the year 2000, the Geological Survey predicts that the United States will be completely dependent on imports for 12 commodities, more than 75% dependent on imports for another 19, and more than 50% dependent on imports for a further 26 commodities.

acting as partners: KEWA and UHDE.

● only the first two reactors of the eight planned are to be delivered on a turnkey basis by KWU, with work starting on one in 1976 and the other in 1978. Nuclebras and KWU are going 75-25 in a nuclear engineering company, while the Austrian firm Voest takes part in a nuclear components company.

If there is thus a vast commercial pay-off for the Federal Republic, there is also a longer-term advantage of capital importance. The Federal Republic will have guaranteed access to uranium—and a guaranteed source of enriched uranium just at the time the squeeze starts operating. This means escaping from the present dilemma of having to rely on either the USA or the Soviet Union to provide the enriched uranium. The uncertainties of this were underlined earlier this year

when supplies to the European Community were halted without warning or consultation, formally because of inadequate security. US pressures on economically sensitive areas like this are ill-received even by such loyal "Atlantic" partners as chancellor Helmut Schmidt—and the calls on the Federal Republic to drop the Brazilian deal tended to confirm the suspicions of those who had opposed German signature of the Non-Proliferation Treaty on the grounds that it would be used by US interests to hinder the Germans on world markets.

Thus the German-Brazilian deal is another step towards a changed pattern of world influence in terms of economic, commercial and technological weight and independence. The strongest of the western European industrial countries has linked hands with the country which is potentially a "first-league" member. □



## Plant history in the Soviet Union

from Vera Rich

THE meeting of the Twelfth International Botanical Congress, which opened in Leningrad on July 6, has yielded a number of interesting reports from Russian botanists. In the field of palaeobotany, Leningrad palaeobotanist Boris Timofeev reported finding fossils of unicellular plant life in a 6-km core sample taken from the Ukrainian shield. This find, he claims, should be dated at 2,000 million years BP. It was also stated that this discovery will be important in the dating of strata of ancient rock, and that it will be of particular importance in oil and gas prospecting—though this claim seems somewhat obscure, since fuel deposits are conventionally attributed to the Carboniferous and not the Palaeozoic Period.

Also in the field of dating, a team of biologists and archaeologists have developed a "unified dendrochronological scale" for estimating climatic changes in the last 1,200 years. More than 8,000 specimens of wood (mostly pine and fir) were used, from the surviving remnants of bulwarks, fortresses, log-built palaces, water conduits and the wooden pavements which were a notable feature of a number of major cities of the state of ancient Rus', notably Novgorod. A reliable time scale for eastern Slavonic history begins only in 988 AD with the conversion to Christianity of the Principality of Kiev, and the exact claim that the most ancient logs used (from sites at Pskov) date back to 770 AD shows rather too great an apparent precision. Equally puzzling is the claim that the data collected not only refer the growth of trees to the general picture of climatic change, but also to "events of cosmic origin". In particular, retarded tree growth is said to be correlated with chronological data on the flares of supernovae, intense meteorite showers and "dimness" of the Sun. This, however, raises the problem of how the original dating is arrived at. Although presumably certain wood specimens can be clearly attributed to the dateable destruction of a given city (and in at least one case, the sack of Novgorod by Vseslav "the Wizard" in 1066, there is an exact coincidence with a cosmological phenomenon—the appearance of Halley's comet), the question how long the wood had been in use when destroyed remains open. If the dating involved is simply carbon-14 measurements, then the margin of error is surely too great to effect a definite correlation to a definite historical event. One suspects that, at some point, some circular argument has entered into the discussion.

Meanwhile, in the field of modern botany, an engineering team reports the development and use of "climatrons"—artificial climate machines with special light bulbs to stimulate different spectra of solar radiation, and special humidity and heat control systems, which provide a short cut to the evolution of new varieties of cultivated plants.

One such new variety (whether evolved in a climatron or not is unclear) is the splendid grass reported by Aleksandr Fedorov, which is intended as the cattle feed of the future and which grows to a height of some 5 m. In his message of greetings to the conference, Mr Kosygin, Chairman of the Council of Ministers of the USSR, expressed his view that on botany, "to a considerable degree, are based achievements in many branches of practical human activity, including agriculture . . ." Certainly, at first glance, the 5 m grass must rank as such an achievement. Presumably it is intended for silage, rather than direct grazing. Even so, the practical problems involved in its production on a commercial scale may well prove somewhat of an embarrassment to stock rearers, haymakers and the manufacturers of agricultural machinery.



● THE grainlands of the Steppe have been a potential cause of political tension throughout their history—indeed, there are those who would attribute the underlying cause of the Trojan war to the need of the Greek tribes to break through the Trojan blockade of the Dardanelles to the wheatlands north of the Euxine. The nineteenth century brought, in Britain, the vexed issues of the corn laws and free trade, both of which assumed an inexhaustible source of wheat in the then Russian empire. In the twentieth century, Hitler envisaged an annexed Ukraine as the breadbasket of his Thousand-Year Reich, relying, presumably on the traditional picture of its fertility, rather than on the events of recent history. For, under Soviet rule, grain production has consistently failed to reach the

planned target figures, and, in spite of the great drive to open up the virgin lands of Siberia to the plough, Soviet agriculture is no longer able to satisfy its home market, and this year, once again, must buy some 10 million tons of grain from abroad.

According to the Soviet press, most of the blame is to be placed on administrative difficulties—farmers so busy with paperwork on crop returns that they have no time to go about their primary business of cultivating and harvesting, machines standing idle for lack of spare parts or maintenance, or the occasional "wrecker" (a recent issue of *Pravda* reports that collective farmers not infrequently feed loaves of intended for human consumption to the livestock which they are allowed to tend as their personal property in their spare time). This is, however, a relatively easy solution to offer. The real situation, however, is more complex, and involves not only misapplication of the administrative process, but the whole Soviet system of planned agriculture.

In spite of the division of the Soviet Union into Union and Autonomous Republics, and (on a somewhat different basis) into economic regions, all planning in agriculture, as in all other types of production, originates in Moscow, and, it would seem, is often carried out with little regard for local conditions. Stalin once, in speaking to Churchill about the "bad and difficult" time of collectivisation claimed as one of its advantages: "Not only have we vastly increased the food supply, but we have improved the quality of the grain beyond measure. All kinds of grain used to be grown. Now no one is allowed to sow any sort but the standard Soviet grain from one end of our country to the other."

Such a statement, of course, assumes that the "standard Soviet grain" is equally suitable for all the highly diverse habitats of one-sixth of the land surface of the Earth. In fact, a number of standard grain types have been developed for different conditions, and the claims made for them are impressive. A *Novosti* press release issued this year mentions eight valuable varieties of wheat developed at the Institute of Wheat Breeding and Seed Growing at Mironovka, south of Kiev, including, not only winter wheat, but an "insurance" type spring wheat "Mironovskaya-808" which can be used to supplement the grain balance when the winter wheat crop is damaged or frozen. This however raises the question, if winter wheat is at risk in a given area, why not concentrate on spring wheat altogether?

During the "maize craze" of the 1950s this crop was sown indiscriminately throughout the Soviet Union,

including areas such as Byelorussia where it could not be expected to ripen (Khrushchev suggested that in such areas unripe maize could be used as fodder). Even in more southern areas, the crop was introduced without proper arrangements being made for its harvesting, and on one occasion, when Khrushchev was to make a winter tour of Ukraine, the local organisers had no alternative but to flatten with large rollers the ungathered maize cobs that were protruding through the snow!

The ecological problems involved in such centralised planning may also be not fully understood. The forestry conservation measures introduced in the Carpathians last May were intended not only to conserve the diminishing timber resources of the area, but also to restore the water-table of the agricultural areas of western Ukraine. The horizon-to-horizon culti-

vation of the virgin lands, without wind-breaks or chequering of fields, has led to considerable erosion.

Another problem is that of crop-rotation. At the Twenty-Fourth Party Congress in 1971, the Minister of Agriculture, Vladimir Matskevich, stated that "correct" crop rotation had now been introduced on about 87% of arable land, but no details are available on the patterns of rotation involved. Unofficial reports, however, indicate a general tendency away from traditional patterns, and although the use of leguminous plants for nitrogen fixation is highly esteemed, it is chemical fertilisers that are seen as the basis of Soviet agriculture. In his report to the Twenty-Fourth Congress, Matskevich spoke of "mechanization, electrification and chemicalisation" as the basis for the intensification of agriculture, and "popular" articles appear in

the Soviet press on the advantages of chemical fertilisers.

From time to time, the Soviet press reports the triumph of small agricultural teams, working in virtually small-holding conditions, who have been able to increase the output of the land at their disposal by impressive percentages. This can, of course, only be an experimental scheme—its large-scale implementation would undermine the basic theory of Soviet agriculture, collectivisation.

● In the article *Soviet meetings, great and small* (*Nature*, 256, July 17, 1975, page 160), paragraph 6 should have begun: "The case of Dr Giluzman, whose incarceration in a prison camp seems to have been the direct result of his preparation of a report on psychiatric malpractice in the case of General Grigorenko, was raised at last year's Annual Meeting."

In these days of world-wide food shortages and rising food prices, it is hard to believe that a nation whose prairie provinces are often thought of as the breadbasket of the world neglects its agricultural and veterinary medicine schools. Yet that, according to the deans of Canada's 11 faculties of agriculture and veterinary medicine, is exactly what is happening.

Through the Science Council of Canada, they recently published a statement saying the faculties "find themselves chronically underfinanced despite widespread government and public lip-service to the essential importance of food production and the need to apply education and research to its increase."

The "national statement" by the 11 deans went on to say the schools "lack funds for current activities other than teaching students; they do not have enough staff to achieve appropriately small class-sizes; and they have neither the time nor the money to undertake research that is needed and of which they are otherwise capable."

As a result, the deans declared, the faculties are intellectual resources that are being exploited to only a fraction of their potential.

It is not the first time the problems of science in agriculture in Canada have been made public. Two previous Science Council publications identified a lack of coordination in research, particularly between the federal government and the universities. (*Agricultural Science in Canada*, Background Study No. 10, 1970; and *Two Blades of Grass*, Report No. 12, 1971, Information Canada.) Roger Gaudry, recently-retired chairman of the Council, also drew attention to the problems in his Annual Report for 1972-73.

Nor does the neglect of the agriculture and veterinary faculties mean that agricultural research *per se* is neglected in Canada. On the contrary, in 1972-73, Agriculture Canada (the federal government department) spent \$90 million on research and development alone. But by contrast, the 11 agriculture and veterinary medicine faculties received total funding in that year of less than \$50 million—only

## Agricultural research at risk

from David Spurgeon, Ottawa

\$15.5 million of which was for research.

And despite the inauguration in 1972 by the Ministry of State for Science and Technology of a "make-or-buy" policy—under which federal government departments were directed to contract out research where possible—Agriculture Canada remains the only department that spends about 99 per cent of its research budget in-house.

The neglect of agricultural science lies chiefly in the small proportion of total R & D funds spent on agricultural research, and the lack of national planning, in addition to the over-emphasis on in-house governmental research.

"Out of about \$1.5 billion a year spent on scientific and technological research in Canada, agriculture and its related sciences get much less than 10 per cent," says the dean's statement. "For want of staff or funds, needed research projects that promise high 'payoff' simply do not get started."

What makes the situation worse, they continued, is that what agricultural and food research is done is neither planned nor evaluated on a national scale in terms of its need and productivity.

Canada's three veterinary colleges have to turn away highly-qualified applicants for lack of space, the deans said. The four Atlantic provinces together are rarely permitted to place more than six students a year at the only English-speaking institution in eastern Canada, the Ontario Veterinary College.

"The shortage of livestock veterinarians is a national problem for which every Canadian consumer is ultimately paying in increased meat prices."

In certain agricultural disciplines, professionals are also in short supply, to such an extent that government agencies have had to hire a significant proportion of their professional staffs outside Canada.

The deans' report was addressed primarily to the federal government, the provincial governments, and the food industries, according to Dr. Gaudry, who wrote the introduction. But it obviously is hoped many ordinary Canadians will read it as well.

It constitutes a direct challenge to the governments: "We can choose programmed ignorance and increasing risk in our food-production system, resulting from science policy by accident and a failure to understand the relationship between teaching, research and public service in the universities. Or we can choose to develop a fully-articulated set of national agricultural policies and support strategies, in which each sector's distinctive advantages are used efficiently."



# news and views

## Microtubules and membrane topography

from Robert Shields

THE fluid-mosaic model of the cell membrane is rapidly attaining the status of the central dogma of membrane biology. According to this model (Singer and Nicholson, *Science*, **175**, 720; 1972) proteins are free to diffuse in the plane of the lipid membrane and assume random or homogeneous distributions over the cell surface.

More recently, evidence has been produced that suggests submembranous structures such as microtubules and microfilaments may also help to modulate the cell surface molecules.

When mouse B lymphocytes, which have immunoglobulin (Ig) molecules on their surfaces, are stained with fluorescent antibody to mouse immunoglobulin (anti-Ig), several patterns of fluorescence may be observed depending on the experimental conditions. If monovalent anti-Ig is used the cells are stained diffusely, suggesting a random distribution of Ig molecules over the cell. At low temperatures with divalent antibodies a patchy pattern of stain is seen implying partial aggregation of Ig molecules. When the temperature is raised the patches coalesce to form a fluorescent cap at one pole of the cell. Patch and cap formation are not unique to lymphoid cells and quite a wide variety of cells including mouse fibroblasts have been successfully capped using anti-H<sub>2</sub> antibody (Edidin and Weiss, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2456; 1972).

The formation of patches is rather similar to the classical antigen-antibody reaction in solution in that it requires the antibody to be divalent and antigen to be present in multiple sites. But the formation of caps is more complex than formation of patches, in that it is dependent on cellular metabolism, especially ATP synthesis. Patch formation is passive and depends on bivalent anti-Ig molecules collecting Igs by diffusion but several lines of evidence suggest that microtubules and microfilaments may play a part in the translocation of patches to form caps.

Perhaps the strongest evidence for the involvement of microtubules in the movement of cell surface proteins comes from work with the plant lectin concanavalin A (Con A) and B lymphocytes. Native Con A is multivalent and

reacts with the  $\alpha$  methyl-D-mannoside residues that form part of many of the glycoproteins of the cell surface. Con A acts as a kind of honorary antibody and at low doses will lead to capping of its receptors on B lymphocytes. At high concentrations of Con A, however, no patching or capping is observed (Yahara and Edelman, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 608; 1972). High doses of Con A inhibit not only the capping of Con A receptors but also the capping of Ig molecules with anti-Ig. If the cells are treated with colchicine or the temperature lowered to 4 °C, the cells washed free of excess Con A and the temperature shifted to 37 °C, caps will form.

An attractive explanation of these observations was provided by Yahara and Edelman (*Expl Cell Res.*, **91**, 125; 1975). They believe that molecules on the cell surface (such as Ig or Con A receptors) may exist in two interconvertible states, a free state where they are able to diffuse laterally in the lipid matrix, and a bound state where they interact directly or indirectly with microtubular structures inside the cell. Interaction of Con A with its receptors would lead to the binding of free receptors to a lattice of microtubules. The lattice, which is caused to form by high doses of Con A, also interacts with Ig molecules and prevents their diffusion to form a cap. When colchicine or other microtubule-disrupting agents are added, or the temperature is lowered (which also breaks up microtubules) the lattice would break up, allowing free diffusion of surface molecules and the formation of caps. But before this model for receptor modulation can be accepted other possible explanations of the results should be considered.

One possibility is that the binding of Con A to the lymphocytes induces a phase transition of the membrane and decreases the membrane fluidity. This would inhibit the free translocation of Ig and other molecules in the lipid phase. Colchicine and vinblastin at the high doses used by Edelman could conceivably interact directly with the cell membrane increasing the membrane fluidity and permitting receptor movement. Lowering the temperature

might lead to phasing out of the cell membrane into fluid and non-fluid domains and so permit increased receptor movement (Petit and Edidin, *Science*, **184**, 1183; 1974).

These possibilities could be tested quite simply by using fluorescent lipid probes inserted into the cell membrane to measure membrane fluidity by fluorescent polarisation before and after Con A or colcemid treatment. Although the evidence is rather sketchy it seems that binding of lectins to cells is more likely to increase than to decrease membrane fluidity (Toyoshima and Osawa, *J. biol. Chem.*, **250**, 1655; 1975). Furthermore lumicolchicine, an isomer of colchicine which reacts equally well with plasma membranes (Stadler and Franks, *J. Cell. Biol.*, **60**, 297; 1974) but not with microtubules, fails to relieve the Con A-induced inhibition of Ig capping (Yahara and Edelman, *Nature*, **236**, 152; 1973). These experiments would seem to indicate that microtubules are involved in receptor movement.

A further possibility is that the binding of Con A to cells leads to the formation of aggregates on the cell surface that trap Ig molecules and so prevent their diffusion. Since there are about  $1.4 \times 10^6$  Con A receptors and about one thirtieth as many Ig molecules on the cell surface this is a possibility. To counter it, Edelman calculated that even at saturating doses of Con A, Con A receptor complexes occupy only 1% of the cell surface and so are unlikely to interfere directly with the free diffusion of Ig molecules (Edelman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1442; 1973).

A related and more plausible possibility is that, since Con A will interact with many different glycoproteins through  $\alpha$  methyl-D-mannoside residues, Con A may in fact directly crosslink Ig molecules at the cell surface, rather than through some submembranous microtubular structure. This idea was given credence by the observation that Con A was able to cap Ig molecules without the need for anti-Ig. Furthermore this co-capping takes place even in the presence of colchicine or at 4 °C, conditions that should disrupt microtubular structures. Con A also

induced co-capping of other molecules, such as the H-2 and  $\Theta$  antigens in splenic lymphocytes, without recourse to use of the specific antibodies. In the light of these results it was vital to show that the Con A-induced inhibition of Ig motility was not an uninteresting artefact due to crosslinking at the cell surface but that microtubular structures were involved.

To do this Yahara and Edelman took advantage of the relative difference in size between the B lymphocyte and blood platelets (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1579; 1975). They conjugated the small platelets with Con A and studied their interaction with splenic lymphocytes. The much larger lymphocytes could bind several of the platelets through the conjugated Con A, and the platelet distribution was random over the lymphocyte surface. The idea was to complex only a small proportion of the total Con A receptors of the lymphocytes and to see if Ig movement was still restricted. Staining the platelet-treated lymphocytes with fluorescent Con A revealed that only a fraction of the lymphocyte Con A receptors were bound to the platelets; furthermore the Con A receptors not bound by the platelets were distributed diffusely over the lymphocyte surface. The binding of more than ten platelets per cell was sufficient to prevent anti-Ig-induced capping of Ig molecules, showing that Ig redistribution was blocked by the occupation of only a fraction of the Con A receptors. When the lymphocytes with their bound platelets were treated with colchicine they showed polar or cap-like distribution of the platelets on their surfaces. But when these colchicine-treated cells were examined with fluorescent anti-Ig for the distribution of Ig molecules, no co-capping of Ig molecules with the Con A platelets was seen: the motion of bound Con A receptors and Ig molecules was now independent. This provides the strongest evidence that Con A receptor modulation of Ig on the surface of B lymphocytes involves microtubular structures and is not an artefact generated by crosslinking of Ig molecules to Con A receptors on the cell surface.

The evidence for the involvement of microfilaments in the movement of molecules in the cell membrane is even more indirect and depends on the use of the drug cytochalasin B. This agent has had a rather poor press recently and it is not clear how it interferes with microfilament function. A further complication is that different preparations of this drug have different activities. What is fairly clear however is that cytochalasin B will interfere with cap formation and in the right condi-

tions will lead to an 80% inhibition of cap formation induced by anti-Ig. When used in conjunction with colchicine, cap formation is almost completely blocked. The addition of cytochalasin B to cells that have already capped leads to a dissolution of the capped molecules (de Petris, *Nature*, **250**, 54; 1974).

These findings suggest that microfilaments as well as microtubules have a role in the movement of molecules on the cell surface. What is required now is some more direct evidence of the linkage of molecules on the cell surface to microtubules and microfilaments in the cytoplasm.

## Another overexploited fishery

from our

*Marine Vertebrate Correspondent*

OVEREXPLOITATION of fishery resources is no new phenomenon. Examples range in emotive potency from the state of whale stocks, through the decline in numbers of the Atlantic salmon, to the humble North Sea herring and the Californian sardine. Developments in fishery technology, aimed at improving the catch-effort ratio, have generally produced increasingly efficient means of exhausting the stock.

There is no worse offender in this respect than the Japanese high-seas fishery for tuna which during the last two decades exhausted the stocks in the southern Indian Ocean and the western Pacific, before transferring its operations to the Atlantic and the eastern Pacific Oceans. The consequences of this fishery on the billfishes (sailfish and marlins) of the eastern Pacific are discussed in a recent paper by Talbot and Wares (*Trans. Am. Fisheries Soc.*, **104** (1), 1-12; 1975).

By 1963 the Japanese fishery had expanded to 10° north latitude, offshore of Central America, and heavy catches of yellow-fin tuna (*Thunnus albacares*) and striped marlin (*Tetrapturus audax*) were made. In time it spread further north and the marlin became the major exploited species. The success of this fishery resulted in increased fishing effort and in 1964 the catch of this one species approached 300,000 fish. Thereafter the catch declined until 1967, reached a high point in 1968 of around 320,000 fish, but dropped dramatically the next year to just over 200,000. A comparable change has been observed in the catches of blue marlin (*Makaira nigricans*) which attained a maximum (nearly 80,000) in 1963 and thereafter declined severely, only increasing slightly (to 30,000) in 1969. The sailfish (*Istiophorus platypterus*) similarly shows an overall decline

from a peak of nearly 400,000 fish in 1965 to 100,000 in 1969.

Talbot and Wares examine the possibility that these changes in catch may reflect abundance variations caused by differing sea temperatures but, except for one area, they find little evidence to support this. They do, however, find clear evidence of overfishing. The first peaks of abundance for each of the billfish species can be related to increased fishing effort, and the decline in catch has persisted in some cases even though the number of hooks fished has increased. Expressed in terms of hook rate (the catch per hundred hooks), it seems that the striped marlin declined marginally from 0.7 to 0.3 between 1968 and 1969, the blue marlin catch fell from 0.35 to 0.05 between 1959 and 1969, while the sailfish catch fell from nearly 3 to 1.2 between 1965 and 1969. Clearly, in the cases of both the blue marlin and the sailfish the decline has been severe and indicates overfishing and depletion of the stocks. And the average weight of these fish has declined, which is also evidence of overfishing.

The reduction in numbers and weight of these billfishes may have considerable consequences for the sport fishery of the western Mexican and Californian coasts. This sport fishery has provided an important contribution to the income of Pacific Mexico since the second world war. Talbot and Wares point out that the reduction in average size of the fish caught has already made it a less spectacular sport. But they suggest that with the declining returns per unit effort the Japanese fishery may well decrease, leaving enough of the smaller billfishes to maintain the sport fishery.

## Exciting days over for reverse transcriptase?

from Karin Moelling

It is five years now since Temin and Baltimore reported the existence in RNA tumour viruses of a reverse transcriptase which can make a DNA copy from the RNA genome. For some time progress on the characterisation of this new type of enzyme filled the front pages of the journals. But results on the characterisation of the enzyme are no longer so spectacular, and have become matters for hard working biochemists.

What do we know about the viral reverse transcriptase? Research was concentrated on the avian viral reverse transcriptase as large amounts of avian viruses can be isolated — and were generously supplied by J. Beard.



We learnt that the purified enzyme transcribes natural viral RNA very efficiently, for which it requires an RNA primer which was identified as a tryptophan tRNA (Dahlberg *et al.*, *J. Virol.* **13**, 1126; 1974). In addition to the RNA-dependent DNA polymerase a DNA-dependent DNA polymerase is involved in the synthesis of the double-stranded DNA product. Furthermore an RNase H activity seems to play a part. All these activities are coded for by the viral genome as was shown with avian viral temperature sensitive (ts) mutants (Verma, Mason, Drost and Baltimore, *Nature*, **251**, 27; 1974).

### Enzyme from murine virus

One would have expected it to be simply a matter of routine to generalise the knowledge about avian viral reverse transcriptases to those of viruses from other species. This was, however, not the case. Mammalian viral reverse transcriptases could copy synthetic templates such as poly (rA). oligo (dT)—but did not seem to be able to carry out what they were given their name for doing, the reverse transcription of exogenous viral RNA into DNA! The RNase H seemed to be absent as well.

So the initially fast progress was followed by a series of negative results. But the results obtained with the avian enzyme were so well established that nobody could have seriously questioned their relevance also for mammalian viral enzymes. Therefore it did not come as a surprise when finally three detailed analyses showed that murine leukaemia viral reverse transcriptases had all the properties of avian enzymes: natural RNA transcription, presence of RNase H in a single polypeptide with the polymerase activities (in two of the three reports) and proof of its viral, not cellular, origin on the basis of its exonucleolytic mode of action (Moelling, *Virology*, **62**, 49; 1974; Gerard and Grandgenett, *J. Virol.*, **15**, 785; 1975; Verma, *J. Virol.*, **15**, 843; 1975).

What was the problem with the mammalian viruses? Apart from inadequate assay conditions used in some cases the starting material seemed to be important. Mammalian viruses may be more sensitive to manipulation and the enzyme seems to be inactivated more readily.

As far as the RNase H is concerned, there was still a confusing matter left to be settled: Gallo's group reported that they could separate the RNase H from the viral reverse transcriptase (Wu, Sarngadharan, and Gallo, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1871; 1974) which suggested that it could be a host cell contaminant.

This problem seems to have been nicely clarified by the latest paper of Gerard and Grandgenett (*J. Virol.*, **15**, 785; 1975). Like good diplomats they show that both results are correct: there is one RNase H which can be removed from the transcriptase; another (the viral one), which cannot, is present in smaller amounts and may have been missed by Gallo's group. The one which can be removed is very small and looks to me on first sight similar to the 'mini'-RNase H recently described as one of the cellular RNase H molecules in calf thymus cells (Buesen and Hausen, *Eur. J. Biochem.*, **52**, 179; 1975). It remains to be shown that the removable RNase H is really of cellular origin. The ultimate proof of the presence of viral RNase H in the single polypeptide murine viral reverse transcriptase molecule must come, as with the avian enzyme, from viral ts mutants.

The enzymatic properties of the reverse transcriptases of viruses from avian and at least one mammalian species, the murine one, seem quite similar. But in spite of their enzymatic relatedness they look structurally quite different: the avian reverse transcriptase has been described as a two polypeptide complex of molecular weights 110,000 and 70,000, called  $\beta$  and  $\alpha$  respectively, and the murine viral reverse transcriptase consists of a single one. But there are also reports (Verma *et al.*, *J. Virol.*, **13**, 1075; 1974; Gerard and Grandgenett, *J. Virol.*, **15**, 785; 1975) which offer two or even three subunits. The number of subunits may not be very relevant — except if some of them are host factors which supply functions missing in the single viral subunit. Indeed the purified viral enzymes are deficient in properties which they must exhibit *in vivo*: *in vitro* they are only capable of synthesising short DNA sequences which are not representative of the total genome.

I do not believe, however, that some of the described subunits are cellular factors. It seems much more likely that the smaller ones are degradation products of a larger one. So far, this has only been shown in the case of the avian viral reverse transcriptase whose larger polypeptide spontaneously undergoes transition to the smaller one, a process which can be stimulated by mild protease treatment (Moelling, *Cold Spring Harb. Symp. quant. Biol.* **39**, 969; 1974). Tryptic peptide pattern analysis by Gibson and Verma nicely confirms this relationship between  $\beta$  and  $\alpha$  (*Proc. natn. Acad. Sci. U.S.A.*, **12**, 4991; 1974). To finally prove that the larger molecule  $\beta$  is the actual reverse transcriptase it must be isolated and

characterised — a difficult task, as  $\beta$  is very labile. Proteolytic degradation products may still retain some enzymatic properties, especially response to synthetic templates — as is the case with the isolated avian breakdown product  $\alpha$ .

If the  $\beta$  subunit is the real avian viral transcriptase, it might correspond with the single polypeptide murine viral enzyme. But this is so far unproven. Two properties of the murine enzyme bear a strong resemblance to those of the  $\alpha$  subunit: its low affinity for templates and its rather low response to natural RNA templates, which can be dramatically stimulated by addition of primers. It may therefore very well be that the actual mammalian viral reverse transcriptase has not yet been identified.

Perhaps the question of what the viral reverse transcriptase looks like cannot be answered by isolating the enzyme from the virus. One may have to look instead at the intracellular state of the enzyme. This has been done by Gallo's group (Mondal, Gallagher and Gallo, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1194; 1975), who found interesting size differences between the reverse transcriptase from virus-producing cells and that of extracellular virus particles: the intracellular one is larger. It is not yet clear whether the high molecular weight class is an aggregate of the low molecular weight form and there is no evidence to correlate the two size classes with the avian  $\beta$  and  $\alpha$  molecules.

One remaining problem is the small size of the DNA product of the reverse transcriptase. Baltimore and Duesberg recently made some progress here by increasing the deoxyribonucleotide concentration in the *in vitro* reaction, but the large amounts of radioactively labelled precursors required will be too expensive for normal use. Perhaps addition of cellular ligases will improve the size of the DNA product. Another important achievement would be the synthesis of a DNA product which represents all regions of the RNA template equally well—not just a small region in large amounts.

Furthermore the role of the RNase H needs to be shown. Does it remove the RNA in order to allow double-stranded DNA synthesis to occur? Such a mechanism was suggested soon after its discovery and is now also incorporated in the Cooper and Wyke replication model (*Cold Spring Harb. Symp. quant. Biol.*, **39**, 997; 1974). But other models, requiring a complicated interaction between cellular and viral enzymes, have been proposed and recently supported (Leis *et al.*, *J. Virol.*, **15**, 484; 1975). Cooper and Wyke point to another interesting question: How

is the RNA primer removed from the RNA template to allow transcription of the anti-primer region?

None of these unsolved problems has affected the great usefulness of the viral reverse transcriptase in identifying the presence or involvement of RNA tumour viruses—particularly in human neoplasias. One still hopes for a specific inhibitor in order to test its effect on tumour growth—inhibition of reverse transcription is the only known virus-specific step which would not affect normal cellular functions. Here H. Temin might object: he believes a reverse transcriptase plays a part not only in viral replication but also in normal cell development, for example in gene amplification. So far there is only weak evidence in favour of this exciting idea—but Temin was right once before!

## Androgens converted to oestrogens

from our Steroid Biochemistry Correspondent

ALMOST 20 years ago it was demonstrated that androgenic steroids could be converted to oestrogenic ones even in the absence of steroid-producing endocrine organs: administration of testosterone to adrenalectomised ovariectomised women increased the urinary excretion of oestrogens. Recent studies have been concerned with the sites where such an aromatisation may occur and with the possibility that even a low level of conversion has an important effect at those sites.

Most tissues, including liver, seem able to bring about this conversion. Breast fat can form oestrogens (Nimrod and Ryan, *J. clin. Endocr.*, **10**, 367–372; 1975) and both abdominal and axillary fat can convert androstenedione to oestrone. This conversion by adipose tissue is surprising as one might expect the lipophilic steroid to be protected from the active enzymes by dissolving in the fat. Even though the rate at which oestrogens are produced *in vitro* by adipose tissue is very low, the large proportion of adipose tissue in the body (up to 30%) may make this tissue an important source. Oestrogens may have an important role in the aetiology and treatment of breast cancer; a large proportion of tumours are known to be oestrogen dependent and contain oestrogen receptors. But fat from normal breast tissue and that from subjects with cancer seems to have identical aromatising ability. Although oestrone is the main product formed, small amounts of oestradiol are also obtained.

Oestrogens may have a role in hair

growth and the presence of the aromatising enzyme system in anagen hair roots has also been demonstrated recently *in vitro* (Schweikert, Milewich and Wilson, *J. clin. Endocr.*, **40**, 413–417; 1975).

Diencephalic tissue from human foetuses (Naftolin, Ryan and Petro, *J. clin. Endocr.*, **33**, 368–370; 1971) or limbic areas of rat brain (Lieberburg and McEwen, *Brain Res.*, **85**, 165–170; 1975) are able to aromatise testosterone or androstenedione but such conversion is negligible when cerebral cortical tissue is used. Whereas the radioactivity associated with oestradiol accounted for less than 1% of the radioactivity in the whole homogenate of the limbic areas of rat brain, in the nuclei oestradiol radioactivity counted for almost half the total. Although oestradiol could have been formed peripherally and taken up by the brain, the evidence would seem to show that oestrogens can be produced from C-19 steroids such as testosterone and androstenedione.

Marked changes in reproductive function can be caused by the administration of either testosterone or oestrogens to neonatal rats. This and other evidence suggests that the conversion of androgens to oestrogens in certain areas of the brain may have important effects on brain differentiation, sexual behaviour and the production of pituitary hormones.

## Dilatancy and aftershocks

from Peter J. Smith

DILATANCY models are generally thought of in connection with either earthquake precursors or man-made earthquakes such as those induced by fluid injection at Denver, Colorado, during the mid-1960s. And though not everyone would agree on precisely how and why the migrating pore fluids account for the phenomena attributed to them, it is clear that if dilatancy is applicable at all it must be applicable to seismic activity extending over at least tens of years (because the precursors of very large earthquakes appear several decades ahead of the main shock). But what part, if any, does fluid flow play in aftershock sequences which also occur within this time scale? The question was first investigated by Nur and Booker (*Science*, **175**, 885; 1972) some years ago. But in having another look at it, Robinson *et al.* (*Geophys. J.*, **41**, 37; 1975) have made an unexpected and encouraging discovery—encouraging because it shows that dilatancy concepts may be adapted successfully to explain phenomena at least one step removed

from those for which they were originally conceived.

The particular sequence studied by Robinson and his colleagues was that of the Inangahua, New Zealand, earthquake of 1968 (magnitude 7.1). The general properties of aftershock sequences, however, are already well known. Thus the frequency of aftershocks immediately after a large shallow earthquake is typically thousands a day and decays with time to perhaps ten or fewer shocks a day 100 days later. Up to a year at least, the frequency is approximately inversely proportional to time and is related to magnitude (of the aftershocks themselves) through the well known Gutenberg–Richter equation in which  $\log$  (frequency) is proportional to magnitude with a constant of proportionality  $b$  (the so-called  $b$  value). Geographically, aftershocks are closely related to the fault region of the main shock, and most of them have focal mechanisms similar to that of the main shock.

When Adams and Lowry (*New Zealand R. Soc. Bull.*, **9**, 129; 1971) investigated the Inangahua aftershocks a few years ago, they found them to be no exception to these general rules. The  $b$  value was roughly unity, the shock frequency varied as  $(\text{time})^{-1.05}$ , the epicentres lay within an elliptical area about 45 km  $\times$  25 km extending SSW from near the main shock's epicentre, and both the main shock and most of the aftershocks were apparently due to thrust faulting. But Adams and Lowry only looked at the first 40 days of the sequence. Robinson *et al.*, by contrast, returned to study the sequence 3.6 years after it had begun, by which time the magnitude level had decreased to that of microearthquakes. What they found was that the  $b$  value was still about 1 (having remained constant for more than 3½ years and over a magnitude range of 5), that the shock frequency had continued to decrease with time in the same way (actually as  $(\text{time})^{-1.06}$ ) and that the new epicentres were distributed as the old (the elliptical area had contracted marginally). But the faulting had changed completely, from the dominant thrust movement of the main shock and early aftershocks to the main normal movement of the later aftershocks.

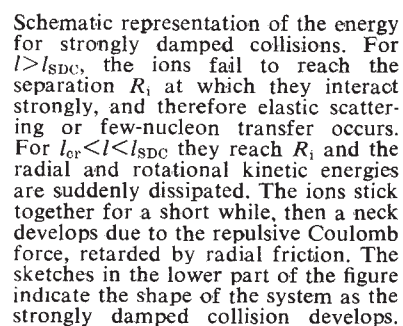
On the face of it, this is surprising. The regional tectonic stress is compressive and thus hardly conducive to normal faulting unless both the stress is reduced to a low level and material is removed at depth. The earthquake and its aftershocks themselves will reduce the stress, but what could remove the material? The obvious explanation, of course, is that the pore fluids which entered the source region before the earthquake have now flowed out again. In general terms, the



## Nuclear friction

This theory has recently been extended by Bondorf, Huizenga, Sobel and Sperber (*Phys. Rev.*, **C11**, 1265;

At slightly lower values of  $l$  the ions interact very strongly; this happens quite suddenly because the nuclear densities rise very rapidly in the region of the nuclear surface. There is thus a critical  $l_{\text{SNC}}$  (strongly damped collision) below which the ions interact strongly. They stick together and nearly all their



We assume that the same thing happens for colliding nuclei. As they begin to separate, a neck forms between them and energy is continually

lost. They finally separate only when their distance apart is  $R_1$ , which is substantially greater than  $R_1$ .

For even smaller  $l$  values, so much energy is dissipated by frictional forces that the nuclei stick together permanently and a compound nucleus is formed. The highest  $l$  value for which this occurs is the critical  $l_c$  for fusion of the two nuclei.

The cross sections for these processes can be expressed in terms of the angular momenta using the expression for the angular momentum

$$l\hbar = mvR = k\hbar R$$

So that the cross-section

$$\sigma = \pi R^2 = \frac{\pi}{k^2} l^2 = \pi \lambda^2 l^2$$

Thus the total cross section for strongly damped collisions

$$\sigma_{SDC} = \pi \lambda^2 (l_{SDC}^2 - l_{cr}^2)$$

and the cross section for compound nucleus formation

$$\sigma_{CN} = \pi \lambda^2 l_{cr}^2$$

This simple picture of the interaction of heavy ions at high energies explains in a qualitative way some of the features that have already been mentioned.

First, it is clear that for  $l > l_{SDC}$ , the energy loss is quite small, while for  $l < l_{SDC}$ , it is very large. This explains the prominent gap in the energy spectrum of the outgoing particles. Second, the formation of a neck means that the ions are separated by more than the sum of their radii when they part, and so will have less repulsive Coulomb energy, again in accord with measurements. Finally, the cross section for the formation of a compound nucleus is much less than the geometrical cross section.

As an indication of the value of  $l$  involved in a typical reaction, Bondorf and colleagues consider the collision of 600 MeV  $^{84}\text{Kr}$  with  $^{209}\text{Bi}$  and find  $l_{SDC} = 250$  and  $l_{cr} \approx 75$ ; showing how large a number of partial waves participate in these reactions. They developed a formalism connecting these parameters of the interaction with the classical angles of emission of the particles at the critical angular momenta, and included a coefficient of friction  $k$  by assuming that the frictional force is proportional to the velocity of the ion. From the angles of the observed peaks in the cross-section they deduced values of  $k$  and found that it varies with  $l$ . In this way they were able to account in a qualitative way for the characteristic angles of emission of the particles losing little energy and of those losing most of their energy.

These ideas have also been worked

out by Gross and Kalinowski (*Phys. Lett.* **48B**, 302; 1974) using the Newtonian equations of motion with frictional forces. They calculated the interaction potential experienced by the lighter of the two colliding nuclei by averaging the nucleon-nucleus optical potential over the density distribution of the heavier, and assumed a predominantly radial form for the friction tensor. With this simple model they were able to account for a large number of experimental fusion cross sections, and their energy variation.

This model will have to be tested for many reactions over a range of energies but already the results are sufficiently encouraging to show the value of applying the classical concept of friction to nuclear reactions. As in the corresponding macroscopic situation, we use the idea of friction to take account in a simple way of very complicated processes we do not attempt to describe in detail, and to build them into a more comprehensive picture of the whole interaction.

## Linking lymphomas with immunodeficiency

from R. T. D. Oliver

"NOWHERE in pathology has a chaos of names so clouded clear concept as in the subject of lymphoid tumours" (Willis, *Pathology of Tumours*, **49**, 760; 1948). Twenty-seven years and three classifications later we understand little more about the basis for the wide range of pathological entities classified as lymphomas. The many immunological deficiency diseases are similarly confusing. At first glance the article recently published in the *Lancet* on Duncan's disease (Purtilo *et al.*, *Lancet*, **1**, 935; 1975) (X-linked progressive combined variable immunodeficiency, to give its full name) seems to add yet another syndrome to the growing list. But more detailed reading suggests that it may provide clues for a better understanding of both immune deficiency syndromes and lymphomas.

Purtilo and colleagues report on studies of a family where six out of eighteen male children in seven branches of the family died of a lymphoproliferative disease involving bone marrow, liver, spleen and CNS. In three of these children it was possible to document an infection with infectious mononucleosis virus (Epstein-Barr virus) at presentation or prior to the onset of the disease and two had histologically proven lymphoma of the gastrointestinal tract and CNS. The authors suggest that infection with the Epstein-

Barr virus in the face of relative inability to respond to that virus triggered the fatal proliferation of lymphocytes (presumably B cells in view of the well-known ability of the Epstein-Barr virus to cause transformation *in vitro* of human B lymphocytes, though the authors fail to describe detailed immunological studies of the patients or their normal relatives). They suggest that the immune deficiency is mediated by a sex-linked recessive gene because exactly 50% of males but no females in the involved arms of the family tree died with the disease. This observation is extremely interesting as to date five out of fourteen previously recognised sub-groups of immune deficiency disease have been shown to have a sex-linked recessive inheritance (Fudenberg *et al.*, *Paediatrics*, **47**, 927; 1971) and it is well recognised that the majority of lymphoma except nodular sclerosing Hodgkin's and nodular lymphomas have an excess of males. But the generally low incidence of familial lymphomas clearly indicates that, if X-linked genes are involved, other genes must be also, as is well illustrated in the family studies of children with acute lymphoblastic leukaemia by Till *et al.* (*Br. J. Haemat.*, **29**, 575; 1975).

There is evidence, from studies in experimental animals, that at least four different classes of immune response gene exist. The first to be discovered was that linked to the H-2 major histocompatibility region, which seems to be involved in initial antigen recognition by T cells (Katz and Benacerraf, *Transplant Rev.*, **22**, 175; 1975), and in resistance to various leukaemia viruses (Lilly and Pincus, *Adv. Cancer Res.*, **17**, 231; 1973). The second class, found in some cases linked to genes controlling immunoglobulin allotypes, regulates quantitative aspects of antibody production (Braun, Eichmann and Krause, *J. exp. Med.*, **129**, 809; 1969). The third class are the sex chromosome X-linked genes which, to date, have only been shown to influence response to T cell independent antigens (Mozes and Fuchs, *Nature*, **249**, 167; 1974). The fourth class recently reported by Jormalainen, Mozes and Sela (*J. exp. Med.*, **141**, 1057; 1975) seems to mediate its effect by some form of suppression of preordained response. If there were this number of different immune response genes in man which could influence the response of patients with lymphoma (even if the disease was induced by a single aetiological agent), it would hardly be surprising that no two lymphoma patients had exactly identical pathology in their lymph nodes.

It is clearly going to be important to be able to detect the different classes of immune response genes in man. A start has been made by classifying lymphomas on the basis of differentiation



T or B cell markers on their surfaces (Brown *et al.*, *Lancet*, **ii**, 753; 1974), and there have been many studies of HL-A antigens in patients with lymphomas; but we are far from understanding the complexity of their interaction with other systems such as the sex-linked locus postulated by Purtilo *et al.* *In vitro* assays, such as that recently reported by Greenberg, Gray and Yunis (*J. exp. Med.*, **141**, 935; 1975) will undoubtedly play an important part in this work.

## Quark soup, with glue

from W. T. Toner

If nuclear matter is made of quarks, so are neutron stars. If the quarks move freely as long as they remain close to each other in the neutron, then they must move freely in the centre of neutron stars where the density is a hundred to a thousand times greater than that of the neutron itself. So argue Collins and Perry in a recent issue of *Physical Review Letters* (**34**, 1353, 1975).

The fractionally charged quark is still the simplest realisation of the abstract logic shown by group theoretical analyses of the spectroscopic patterns made by more than a hundred strongly interacting particles. Although all models have their problems, a remarkably successful fit can be obtained by considering quarks which are light and move freely in a potential well. This might be thought coincidental were it not for the evidence of experiments in which leptons (electrons, muons and neutrinos) scatter from the nuclear matter of neutrons and protons. In violent collisions, these electromagnetic and weak probes measure the degree of concentration of electromagnetic or weak charge in the strongly interacting target:  $\Delta p$  is large and since  $\Delta p \cdot \Delta x \sim \hbar$ ,  $\Delta x$  is small. If the target is allowed to break up, its mass during and after the collision need not be precisely defined and  $\Delta E$  in  $\Delta E \cdot \Delta t \sim \hbar$  can be large,  $\Delta t$  small. Violent inelastic collisions can thus be said to measure the instantaneous degree of concentration of charge. As is by now well known, the data show the large and 'scaling' cross section characteristic of the scattering from quasi-free point charges or 'partons'. The numerical values are most simply understood by assuming that the partons are the same fractionally charged, freely moving quarks that account for the spectroscopy. They seem free so long as we look quickly.

But our best efforts to produce quarks have failed, so how can they be thought to be real? There is no refuge in the abstractions of the algebra of currents since any theory which describes the data turns out to have the character of a free field

theory, and free fields mean free particles, or quasi-particles at the least.

The theorist is required to invent a glue to bind the quarks with the property that if they try to move too far apart the restoring force becomes very large, possibly infinite. But the closer they get together, the weaker it becomes: the quarks are asymptotically free. Such theories are being developed and some even have the very desirable properties of gauge invariance and renormalisability. Particle physicists are so used to forces which increase at short distances that they find such a behaviour peculiar and the talk of 'quark confinement bags' hard to swallow, although analogies in other branches of physics are not hard to find. Consider the electrons in a metal, liberated from the strong binding forces of their own atom by the proximity of others and able to migrate under the influence of infinitesimal potential differences until they reach the surface, where they meet a potential wall several volts high. Closer to home, there is the independent particle model of the nucleus.

If the long-range force binding quarks in elementary particles is not just large, but infinite, so that quarks can never be observed directly, it will be extremely difficult to verify an asymptotically free theory. The predictions are likely to depend as much on the values of the parameters as on the essential correctness of the ideas. For example, the simple prescription for electron-positron annihilation that the cross section is given by the sums of the squares of the quark charges is only valid in the asymptotic region, and where might that begin? It would be invaluable to have independent confirmation of the asymptotically free quark picture from a qualitatively different situation.

Collins and Perry argue that in the enormously dense matter at the centre of

neutron stars the strongly interacting neutrons must overlap to such an extent that the strong, long-range forces—whatever their precise nature—will be screened and the quarks will no longer be confined to one group. They will interact rather weakly with each other and should move freely as if in a superdense gas or 'soup'. Since many of the properties of gases depend on correctly counting the number of independent states, their results differ from those of theories in which the neutrons and other composite strongly interacting particles make up the soup. Because the short-range forces which remain are weak, they should be calculable by perturbation (power series) methods. Detailed examinations of cosmological and astrophysical questions will be made in future papers.

## Rabies

from Arie J. Zuckerman

THE two unassociated recent deaths from rabies in London underline the fact that even with modern intensive medical treatment there is little chance of recovery. Rabies is not declining in prevalence; on the contrary it is spreading, especially among wild animals (*Nature*, **242**, 228; 1973). The current outbreak in Europe is advancing in France along a front nearly 800 km long at a rate of about 30 km a year (*Nature*, **251**, 663; 1974).

Rabies virus is classified as a member of the rhabdovirus group, which includes a diverse collection of bullet-shaped RNA viruses from mammals, reptiles, fish, insects and plants. The virus particles contain two distinct major antigens, a glycoprotein from the virus membrane and an internal nucleoprotein antigen. The glycoprotein antigen elicits the formation of virus-neutralising antibodies which afford protection in animals against subsequent challenge with rabies virus. The same antibodies are probably also protective in man (*WHO Techn. Rep. Ser.*, No. 523; 1973). Complement-fixing and precipitating antibodies to the nucleoprotein are devoid of virus-neutralising activity. The role of cell-mediated immunity in protection is as yet undetermined, but it may be crucial in the exposed individual.

Rabies virus is transmitted by contact, commonly through a bite. Human infection is usually acquired by bite but infection by aerosol has resulted from natural and laboratory exposure (the latter during vaccine preparation), and there is also some evidence of oral infection and aerosol contact in animals. The incubation period is 3 to 8 weeks. The virus spreads from the site of introduction to the central nervous system along the peripheral nerves.



### A hundred years ago

In an article in the July number of *Symons's Monthly Meteorological Magazine*, on the French floods, is an interesting calculation which will give Londoners some idea of what a "flood" means. Supposing we had a flood in the Thames, it would cover on the south bank, the whole of Battersea Park, Lambeth, Southwark, Bermondsey, and Deptford; and on the north bank, Fulham, Chelsea, Brompton, Belgravia, Westminster, and St. James's Park; while, as for the new embankment, a steamer might ply over the top of it.

from *Nature*, **12**, 261; July 29, 1875.

Following infection of the central nervous system the virus may multiply in the salivary glands, in the kidneys and elsewhere and in some species in the muscle and the lungs.

Rabies is enzootic in all countries, except Australia and Antarctica, existing in two forms: urban rabies, propagated principally in dogs, and rabies in wildlife. Wild carnivores appear to be the main transmitters of epidemic and endemic wildlife rabies and methods of control have been reviewed in detail elsewhere (WHO Report, *loc. cit.*). Some progress has recently been made in the treatment and prevention of rabies in man. Ideally, before instituting treatment it is important to determine whether the biting animal was vaccinated and whether the animal was rabid. Local treatment of the wound is extremely valuable, especially when applied very early after exposure. This is followed by the combined administration of anti-rabies serum, preferably of human origin, and active immunisation. In many countries an immunisation schedule of 14 to 21 daily injections is being used and booster doses are essential when combined antiserum-vaccine treatment is employed. A rabid patient should be isolated and treated in an intensive medical care unit.

The vaccines which have been used are of two types: those derived from the brain of adult or newborn animals and those derived from avian non-nervous tissue. Vaccine prepared from a Pasteur or similar strain of fixed virus grown in duck embryos has been developed in an attempt to eliminate the hazards associated with the brain tissue vaccine, which include neuroparalysis. Some progress has also been made in the purification of nervous tissue vaccines by extraction with fluorocarbon and other treatment. But even more promising are the vaccines produced in cell cultures. In the USSR and Czechoslovakia the Vrukovo-32 strain has been grown in primary hamster kidney cells, in France vaccine has been prepared in primary foetal bovine kidney cultures and in the USA and France an inactivated vaccine is being produced in human diploid cells with a Pasteur-derived PM strain adapted to cell culture. Three injections of this vaccine, given at intervals of 3 to 4 days in previously non-immunised subjects, elicit a neutralising antibody response which is much greater than that induced by 12 to 14 daily injections of either brain tissue or duck embryo vaccine (WHO Report, *loc. cit.*). Indeed evidence of studies to date indicates that cell culture vaccines offer distinct advantages over existing vaccines of nervous tissue or avian origin, from the points of view of both safety and potency. Clearly careful assessment and evaluation of the newly developed cell

culture vaccines for man must continue. Pre-exposure immunisation of persons who run a high risk of repeated exposure, such as veterinarians and field naturalists, is highly desirable. Such immunisation consists of three injections of a potent vaccine at 5- to 7-day intervals followed by a booster injection a month later and subsequently at intervals of 1 to 3 years. Prophylactic vaccination of dogs and cats and other species where appropriate is most important, as are local administrative programmes and international control of transfer of animals. But the control of rabies in wildlife continues to challenge man's ingenuity.

## Nuclear reactor in the jungle

from S. A. Durrani

An international symposium on the Oklo phenomenon was held at Libreville in Gabon on June 23-27, 1975, under the joint auspices of the International Atomic Energy Agency (IAEA) and the French Atomic Energy Commission (CEA), and sponsored by the Government of Gabon.

NATURE, it would seem, had anticipated man by something like 1,800 million years in bringing about the first self-sustained nuclear chain reaction on the Earth. And, contrary to common belief, it was not in the squash court of the University of Chicago in December 1942, but in the wilds of what is today the Republic of Gabon at a place called Oklo that this fantastic phenomenon took place.

The history of the discovery of the phenomenon, as it unfolded during the symposium, is briefly as follows. In June 1972, a team working under the direction of Dr H. V. Bouzigue at the CEA service laboratory at Pierrelatte in France noticed a marked anomaly in the abundance of the uranium-235 isotope ( $0.7171 \pm 0.0010$  in atomic per cent instead of the normal  $0.7202 \pm 0.0006$ ) during the certification of a secondary standard of  $UF_6$  by the gas diffusion method. Later, much larger depletions of this isotope were discovered (down to 0.621%, and eventually to 0.296%  $^{235}U$ ) in uranium samples from this source, which was traced back to the Oklo deposit. First positive proof of the hypothesis that a natural chain reaction was responsible for the depletion of the fissile component was furnished by Mme M. Neuilly and co-workers of CEA through the measurement of the ratios of fission-product rare earths detected in the ore by the spark source mass spectrometry technique. Two simul-

taneous submissions by the above two groups on September 25, 1972, to the Proceedings of the Academy of Sciences, Paris, announced the discovery and the proposed explanation of this remarkable phenomenon. It was pointed out that at the time of the reaction the natural abundance of the relatively fast-decaying  $^{235}U$  isotope was more than 3%. This natural 'enrichment', helped by the moderation of the fission neutrons by the water content of the soil which enhanced their fission efficiency, and possibly by the relative absence of neutron-absorbing elements in the surroundings, allowed a nuclear chain reaction to develop. It is perhaps worth mentioning that such a natural chain reaction had already been predicted, on theoretical grounds, by several scientists, notably by P. K. Kuroda as early as 1956. The scientific secretary of the symposium, Dr R. Naudet of CEN, Saclay, has since late in 1972 been leading the 'Franceville Project' established by the French CEA to investigate the phenomenon, and has done a great deal to promote its study internationally.

One of the questions discussed at the conference (for example by E. Roth of CEN, Saclay) was whether the Oklo phenomenon was unique. The general feeling was that such propitious circumstances must have occurred elsewhere on the Earth. The timing ( $\sim 1,800$  Myr ago) may well have been optimal. The role of organic matter in producing 'super-concentrations' of uranium in the Franceville basin was delineated by J. Connan (SNPA-Pau, France). At earlier times, organic matter may not have been present in sufficient quantities, whereas more recently the  $^{235}U$  component would have diminished. One intriguing possibility being discussed in the corridors of the conference was whether the radiation accompanying Oklo-type reactions over the globe could have played a role in the furthering of the evolutionary process. (It is believed by some palaeontologists that the origin of mitotic, nucleated cells lies somewhere between 1,500 and 2,000 Myr ago.)

The fact that most of the fission products seem to have stayed put over  $\sim 1,800$  Myr has great relevance to present-day waste disposal problems in the field of nuclear reactors (and nuclear explosions). These implications were reviewed by R. D. Walton (Division of Waste Management and Transportation of USERDA) and by C. Fréjaques (CEA). The latter pointed out that  $^{239}Pu$  seems to have hardly moved at all during its half-life of  $\sim 24,000$  yr. Such conclusions, if confirmed, would be of profound importance in the future development of nuclear energy, both peaceful and destructive.



# review articles

## Archaeological evidence of environmental change

G. W. Dimbleby\*

*Pollen analysis, particularly combined with data from other animal, vegetable and mineral remains on archaeological sites, can yield much information on climatic conditions and the development of agriculture in prehistory. There remain, however, considerable problems of interpretation where the indications from different sources are at variance.*

It is now commonplace to read accounts of changing environmental conditions over periods of thousands of years, sometimes even going back into the earlier stages of the Pleistocene. This is largely due to the great development of pollen analysis, combined in later periods with radiocarbon dating, which has now been applied in many different parts of the world and to many different types of deposit. Evidence of climatic change is also indicated by other features such as change in sea levels or invertebrate faunas. In the more remote past these changes could only have been due to changes in climate and other secular factors determining the environment.

In later times, however, and especially in the past 10,000 years, man was operating within the landscape, using powerful ecological tools such as fire or agricultural practices. Some effect of these activities is often detectable in the pollen record of lake deposits or peat bogs; for instance, a reduction of tree pollen relative to herb pollen<sup>1</sup>, or the appearance of weeds of arable land<sup>2</sup>. There are circumstances in which it is not easy to distinguish secular changes in the environment from the anthropogenic effects. For example, in the past 2,000–3,000 years of the postglacial period in Britain there has been a proportionate increase in the representation of birch (*Betula*) in the tree pollen totals<sup>3</sup>. This could be due to a progressive cooling of the climate, to a progressive acidification of soils, or it could be due to the influence of man in creating a more open landscape, and in particular with his use of fire to do so. What can rarely be told from pollen analyses is where changes were taking place and with what intensity.

It is here that ecological investigation of archaeological sites themselves can often fill in the picture. Once man started constructing earthworks, that is, from the Neolithic onwards, in doing so he was burying a sample of the contemporary land surface and so preserving it for posterity<sup>4</sup>. Even before then, in the Mesolithic, he sometimes created conditions which produced the same effect: wind blown sand resulting from forest destruction on occasion buried a contemporary land surface<sup>5</sup>. These buried surfaces, and to a lesser degree the material of the earthworks themselves, can be studied by some of the conventional methods, appropriately adapted, and also by other methods which are not applicable to the aquatic situations which are the usual source of evidence of past conditions.

Before I go on to consider these applied techniques in more detail, one important point must be made. In a peat bog,

preservation of pollen and other organic remains can be very good; moreover, such remains are well stratified, though perhaps not always as perfectly as is sometimes assumed. A buried land surface, however, can preserve material in no better condition than it was at the time of burial; and if stratification in the contemporary land surface was disrupted, as for instance by the activity of soil fauna, it will be preserved in the same disrupted state.

### Pollen analysis

The value of pollen analyses of buried soils must depend on whether pollen analysis can be usefully applied to terrestrial soils. There is now quite an extensive literature on this subject<sup>6–10</sup>, and it seems quite clear that acid soils can not only provide a surface humus layer which contains a contemporary pollen spectrum, but that the mineral soil beneath contains a record in broad terms of the vegetation history of the site, brought about by the progressive downwash of the older pollen into the deeper layers of the soil. This record may cover hundreds or even thousands of years.

Base-rich soils may contain no pollen, or at best small quantities. Such soils are often actively worked by earthworms and other soil animals, so that stratification is destroyed. It can be argued, however, that microbiological breakdown of pollen is rapid, so that all the pollen in the solum is more or less coeval. If so, the pollen of all the samples counted can be totalled to give a single pollen spectrum<sup>11</sup>.

Using pollen analysis of buried soils it has been possible not only to show the progressive clearance of areas such as the North York Moors, but within any one area to show what types of land use were in operation in the various periods<sup>12</sup>. Even on calcareous soils clear evidence of open country in the early Neolithic has been obtained by this method<sup>11</sup>, and it seems that it remained open from then onwards.

The pollen analyses of buried soils can sometimes be tied in with profiles from adjacent peat or lake deposits, but in other cases there is no apparent correlation. For example Thorley's pollen profile from Lewes<sup>13</sup> indicates that forest cover of the chalk extended until the Middle Bronze Age, yet the analyses of buried soils of Neolithic age may show open conditions<sup>11</sup>. Such discrepancies may arise from the fact that a pollen spectrum from a peat deposit is of a regional character whereas one from a buried soil is essentially local and not necessarily representative of the wider area.

Finally under this heading one should mention that pollen analyses of archaeological sites may contain artificial concen-

\*Institute of Archaeology, University of London, 31–34 Gordon Square, London WC1, UK.

trations of pollen, such as the remarkable high percentages of ivy (*Hedera*) pollen found in some Mesolithic and later sites<sup>14</sup>. Such concentrations are unparalleled in aquatic deposits.

## Other botanical material

Archaeological sites may contain other plant remains, some of which are valuable indicators of environmental conditions. Frequently these are charred, though in special circumstances, such as waterlogged deposits (wells and so on) or the peculiarly anaerobic conditions of Silbury Hill, good preservation of uncharred material may occur. For instance, under Silbury Hill<sup>15</sup>, and in the waterlogged Wilsford Shaft, moss plants were found which were still green. They were readily identified and indicated the existence of chalk grassland turf in Neolithic and Bronze Age times.

In the early 1950s Helbaek<sup>16</sup> carried out his extensive investigation of seed impressions on prehistoric pottery, but since then the development of the flotation technique<sup>17</sup> has increased enormously the direct evidence not only of the crop plants but also of the associated weeds (see, for example, Dennell<sup>18</sup>). In the New World much evidence about early food plants and their associated weeds has come from cave deposits, including human and animal coprolites<sup>19</sup>. Such evidence has been of outstanding importance in the quest for the origins of maize<sup>20</sup>.

Charcoal and sometimes uncharred wood may be found on many archaeological sites. They are of limited value as environmental indicators because wood was a material collected by man for fuel and for constructional purposes. Such finds are therefore usually only of general significance. Nevertheless interesting facts may arise; for example, in spite of the poor showing of *Pinus* pollen in Zone VIIb deposits of Southern England, the charcoal of *P. sylvestris* is recorded in a number of sites through the Neolithic and Bronze Age<sup>21,22</sup>.

Other plant remains may be found using the appropriate techniques. Bud scales have been isolated and identified from waterlogged deposits<sup>23</sup> and grass phytoliths may occasionally be found in large concentrations<sup>24</sup>. The ecological value of such records remains to be seen.

## Animal remains

Vertebrate bones are often an abundant feature of prehistoric sites with a high pH. The larger bones are valuable evidence of man's use of animals and of their structural features, but they are of limited value as ecological indicators—except in so far as domestic animals represent a powerful ecological influence.

The smaller vertebrates—rodents, amphibia, birds—have received relatively little study. These groups present some difficulties of identification, but their potential as environmental indicators is considerable. One source of their remains, found occasionally on archaeological sites<sup>25</sup>, is the regurgitated pellets of birds of prey.

Invertebrate remains offer the greatest promise as environmental indicators, but they can also offer formidable problems of identification. Important results have been achieved with molluscs<sup>26</sup>. In calcareous soils they can provide evidence of changes of environment; for example, the sequence from forest through clearance to grassland has been demonstrated a number of times by Evans<sup>4</sup>. As with soil pollen, their distribution in the soil profile can also give a broad picture of preceding environmental change, though it is not entirely clear how a degree of stratification comes about in a calcareous soil. More work still needs to be done on the ecological requirements of the critical taxa, but it is obvious that they provide a tool of great value.

To an even greater extent is it true that more ecological knowledge is required about the insects. The Coleoptera in particular may be found in considerable numbers and variety of species<sup>27,28</sup> and although groups such as dung beetles may be over-represented, many other species must derive from the

locality and therefore be of particular ecological significance. Insect remains are also proving to be an important feature of urban sites where they can indicate the nature and use of the premises in which they occur<sup>29</sup>.

It has been found that the exoskeletal remains of mites may be preserved in waterlogged archaeological deposits<sup>30</sup> and these, too, may be of considerable environmental significance. Our knowledge of the ecology of many species is still inadequate, but there have been considerable advances in this direction in recent years (see for example ref. 31).

## Soils and sediments

This is a source of information which is rarely, and then only indirectly, available to those working on aquatic deposits. A buried land surface may provide evidence both of the ecological community and of the soil with which it was associated. By comparing the soil profiles buried beneath earthworks of different ages in the same area, it is possible to show the sequences of soil development, which culminates in the present-day soils<sup>12</sup>. It can be demonstrated that on acidic parent materials the clearance of primary forest, followed by pastoral or arable agriculture, may institute a new process of soil genesis, resulting in a different soil—a more acidic and less biologically active one—compared with the mature soil which preceded forest clearance. By combining pollen analysis with observations on the particle-size distribution in a profile it is sometimes possible to show at what stage in the process the earthworms of the original mull humus were eliminated by the soil acidification. In one site at Goodland in Northern Ireland<sup>32</sup>, there is evidence that Neolithic clearance and farming led to soil acidification, loss of structure and eventually peat formation, a conclusion which accords with Moore's recent conclusions from the pollen analysis of blanket peats<sup>33,34</sup>.

Even on calcareous soils there is some suggestion of soil change. Some of the Neolithic soil profiles are very shallow, as though truncated. Evans and Valentine<sup>35</sup> have evidence of hill-wash associated with initial clearance of scarp slopes. Certainly chalk soils eroded massively in late prehistoric times, as evidenced by the dry valley fill<sup>36</sup> and by lynchet formation<sup>37</sup>.

## Multi-disciplinary approach

Though few sites contain material in all the categories described above, most sites contain several different types of evidence. It is important that wherever possible more than one approach should be used. In some instances the different lines of enquiry corroborate each other completely. At Silbury Hill, for instance, the analyses of pollen, molluscs, insects, seeds, mosses and the soil profile all fitted in with a picture of pastoral agriculture in an open landscape. There are circumstances, however, where the different approaches seem to give contradictory results; one may suggest wooded conditions and the other grassland. In some cases these are readily explicable, as at Windmill Hill, where the Neolithic buried soil seems to have been truncated, so that the pollen and mollusc analyses apply to different phases<sup>26</sup>. In other cases no such explanation is obvious<sup>11</sup>, and we have to consider more carefully the nature of the evidence. Pollen grains and snail shells are very different in nature; they get into the soil in different ways and their persistence in the soil depends on different processes. Enough work has been done on both materials to show that consistent results are obtainable with each, but discrepancies show that there are factors we have not yet taken sufficiently into account in building an archaeological interpretation from the analytical data.

Just as conventional pollen analysis was widely used before questions were asked about the relationship of pollen spectra to vegetation in modern environments, so the interpretation of archaeological environmental data is now at the stage of having to pause and consider the validity of the obvious conclusion



indicated by the evidence. We cannot turn to modern equivalents of early farming environments in regions like Europe—they no longer exist. Perhaps this is another area in which experimental archaeology may help us to solve our present problem.

- <sup>1</sup> Turner, J., *Proc. R. Soc.*, **B161**, 343–354 (1965).
- <sup>2</sup> Sims, R. E., in *Quaternary Plant Ecology* (edit. by Birks, K. J. B., and West, R. G.), 223–236 (Blackwell, Oxford, 1973).
- <sup>3</sup> Pennington, W., *The History of British Vegetation*. (English Universities Press, London, 1969).
- <sup>4</sup> Evans, J. G., in *Economy and Settlement in Neolithic and Early Bronze Age Britain and Europe* (edit. by Simpson, D. D. A.), 27–73 (Leicester University Press, Leicester, 1971).
- <sup>5</sup> Dimbleby, G. W., *Proc. R. Soc.*, **B161**, 355–362 (1965).
- <sup>6</sup> Dimbleby, G. W., *J. Soil Sci.*, **12**, 1–11 (1961).
- <sup>7</sup> Guillet, B., *Pollen et Spores*, **12**, 45–69 (1970); **13**, 233–254, 421–446 (1971).
- <sup>8</sup> Munaut, A. V., Durin, L., and Evraud, J. C., *Bull. Soc. bot. Nord France*, **21**, 105–133 (1968).
- <sup>9</sup> Pertsch, R., *Landschaftsentwicklung und Bodenbildung auf der Stader Geest*, (Bundesforschungsanstalt für Landeskunde und Raumordnung, Bad Godesberg, 1970).
- <sup>10</sup> Welten, M., *Veröff. Geobot. Inst. Rübel Zurich*, **33**, 253–274 (1958).
- <sup>11</sup> Dimbleby, G. W., and Evans, J. G., *J. Archaeol. Sci.*, **1**, 117–133 (1974).
- <sup>12</sup> Dimbleby, G. W., *The Development of British Heathlands and their Soils* (Clarendon Press, Oxford, 1962).
- <sup>13</sup> Thorley, A., *Inst. Br. Geogr. Confr.*, Part 5, 47–50 (1971).
- <sup>14</sup> Simmons, I. G., and Dimbleby, G. W., *J. Archaeol. Sci.*, **1**, 291–296 (1974).
- <sup>15</sup> Williams, D., thesis, Univ. Bradford (1975).
- <sup>16</sup> Helbaek, H., *Proc. prehist. Soc.*, **18**, 194–233 (1953).
- <sup>17</sup> Jarman, H. N., Legge, A. J., and Charles, J. A., in *Papers in Economic Prehistory* (edit. by Higgs, E.), 39–48 (Cambridge University Press, London, 1972).
- <sup>18</sup> Dennell, R. W., *J. Archaeol. Sci.*, **1**, 275–284 (1974).
- <sup>19</sup> Callen, E. O., in *Science in Archaeology* (edit. by Brothwell, D., and Higgs, E.), 235–243 (Thames and Hudson, London, 1969).
- <sup>20</sup> Mangelsdorf, P. C., MacNeish, R. S., and Galinat, W. C., in *Prehistoric Agriculture* (edit. by Struver, S.), 471–486 (American Museum of Natural History, New York, 1971).
- <sup>21</sup> Cunington, M. E., *Woodhenge* (Simpson, Devizes, 1929).
- <sup>22</sup> Ashbee, P., *Proc. Dorset nat. Hist. archaeol. Soc.*, **80**, 146–159 (1958).
- <sup>23</sup> Keepax, C., thesis, Univ. London (1972).
- <sup>24</sup> Dimbleby, G. W., *Plants and Archaeology* (Baker, London, 1967).
- <sup>25</sup> Jewell, P. A., *Advmt. Sci. Lond.*, No. 59, 165–172 (1958).
- <sup>26</sup> Evans, J. G., *Land Snails in Archaeology* (Seminar, London, 1972).
- <sup>27</sup> Osborne, P. J., *J. Anim. Ecol.*, **38**, 555–566 (1969).
- <sup>28</sup> Osborne, P. J., *Britannia*, **2**, 156–165 (1971).
- <sup>29</sup> Buckland, P. C., *J. Archaeol. Sci.*, **1**, 303–316 (1974).
- <sup>30</sup> Harrison, S., thesis, Univ. London (1974).
- <sup>31</sup> Wallwork, J. A., *Ecology of Soil Animals* (McGraw-Hill, London, 1970).
- <sup>32</sup> Case, H. J., Dimbleby, G. W., Mitchell, G. F., Morrison, M. E. S., and Proudfoot, V. B., *J. R. Soc. Antiq. Irl.*, **99**, 39–53 (1969).
- <sup>33</sup> Moore, P. D., *Nature*, **241**, 350–353 (1973).
- <sup>34</sup> Moore, P. D., *Nature*, **250**, 439–441 (1974).
- <sup>35</sup> Evans, J. G., and Valentine, K. W. G., *J. Archaeol. Sci.*, **1**, 343–351 (1974).
- <sup>36</sup> Evans, J. G., *Proc. geol. Ass.*, **77**, 347–364 (1966).
- <sup>37</sup> Fowler, P. J., and Evans, J. G., *Antiquity*, **41**, 289–301 (1967).

# Origin of blanket mires

Peter D. Moore\*

*There are three possible causes for the formation of the peat deposits which blanket parts of the British Isles and Scandinavia. Climate and soil maturation each plays a part, but the contribution of human activity now seems to be larger than had hitherto been supposed.*

THE upland, oceanic regions of the western British Isles and Scandinavia have extensive areas covered by deposits of peat, which have developed over a wide range of substrate and topography. The peat blanket is most extensive over plateaux and gently shelving depressions but shallower on or absent from steeper slopes<sup>1</sup>. In depressions, the stratigraphic profile may reveal the presence of former lakes, dating from late-Devensian times<sup>2</sup>, or the remains of damp woodland and carr<sup>3,4</sup> below the blanket peat cover. The age and origin of the blanket peats has been the source of much controversy, but evidence now accumulating links their origin with prehistoric human activity. Until recently, the possible influences of this factor had been underestimated in favour of those of climatic change and developmental processes within soil profiles.

## The age of blanket peats

Early work on blanket peat stratigraphy was carried out by Conway in the southern Pennines<sup>4,5</sup>, where extensive deposits exist. Her conclusion, based on pollen analysis, was that the peats began forming during Atlantic times (about 7,500–5,000 yr b.p.), which seemed reasonable in view of the generally wet conditions which then prevailed. Many of the shelving depressions examined by Conway, however, contained peat-forming woodland communities before the spread of true blanket peat. More recent work by Tallis<sup>7</sup> on the peats of slopes and plateaux suggest a later date, at about the time of the elm decline pollen horizon, although some of his higher altitude diagrams (for example, Featherbed Moss<sup>8</sup>) have basal deposits predating the elm decline.

Pollen analysis of blanket peats from other areas,

summarised in Table 1, show that peat initiation often occurred at about the time of the elm decline, the exceptions being mainly the deeper peats of the southern Pennines. Smith and Pilcher<sup>9</sup> have recently collated radiocarbon dates of the elm decline and other widely used Flandrian pollen zone horizons to examine the basic assumptions of geographical synchronicity of vegetational change, which underlies the general application of the Godwin<sup>10,11</sup> zonation system. They found that the dates of most pollen zone boundaries differ considerably according to the geographic position of the site, whereas the elm decline horizon remains remarkably synchronous at about 5,300–5,100 yr b.p. Whatever the cause of the elm decline, whether climatic change, prehistoric human activity<sup>12</sup> or disease<sup>13</sup>, it seems to be a reliable guide to the date of origin of many blanket peats, at about or just before 5,000 yr b.p. Caution is required, however, in interpreting shallow blanket peat profiles, the base of which may coincide with a secondary elm decline<sup>14</sup>. Profiles from the Highlands of Scotland are difficult to interpret because of the indistinct nature of the elm decline in these areas<sup>15</sup>.

Bartley<sup>16</sup> has published a radiocarbon date of  $5,490 \pm 140$  yr b.p. from the base of a blanket peat profile on Rishworth Moor, West Yorkshire, which coincides with the elm decline. This is slightly older than is normal for this horizon, but peat accumulation is likely to have been slow at the time and the resolution of <sup>14</sup>C dating therefore poor.

The elm decline is itself usually accompanied by palynological indications of human activity and forest clearance, but even in the shallow peat sites, which were initiated later than the primary elm decline, the basal deposits often give indications of considerable human activity<sup>14,17</sup>. It is this coincidence of peat initiation with evidence for human interference with vegetation which makes imperative a reappraisal of the generally assumed climatic origin of blanket mires.

\*Department of Plant Sciences, King's College, 68 Half Moon Lane, London SE24 9JF, UK.

**Table 1** Blanket peat profiles and the relative position of the elm decline horizon to the peat-soil interface

| Area             | Site              | Authors                            | Peat depth (cm) | Distance of elm decline from peat base |
|------------------|-------------------|------------------------------------|-----------------|----------------------------------------|
| Dartmoor         | Taw Head          | Simmons <sup>18</sup>              | 390             | 10 cm below                            |
| Dartmoor         | Okement Hill      | Godwin <sup>18</sup>               | 155             | 5 cm above                             |
| Exmoor           | Chains            | Merryfield and Moore <sup>14</sup> | 290             | 5 cm above                             |
| Mid-Wales        | Plynlimmon        | Moore <sup>19</sup>                | 185             | 10 cm above                            |
| Mid-Wales        | Tywi Valley 5     | Moore <sup>4</sup>                 | 120             | 3 cm above                             |
| Mid-Wales        | Tywi Valley 4     | Moore <sup>4</sup>                 | 65              | 0 cm                                   |
| South Pennines   | Down Head Hill I  | Tallis <sup>7</sup>                | 150             | 0 cm                                   |
| South Pennines   | Down Head Hill II | Tallis <sup>7</sup>                | 185             | 30 cm above                            |
| South Pennines   | Featherbed Moss   | Tallis and Switsur <sup>8</sup>    | 260             | 45 cm above                            |
| South Pennines   | Totley Moss       | Hicks <sup>20</sup>                | 240             | 40 cm above                            |
| South Pennines   | Hipper Sick       | Hicks <sup>20</sup>                | 170             | 15 cm above                            |
| South Pennines   | Ringinglow C      | Conway <sup>5</sup>                | 600             | 50 cm above                            |
| South Pennines   | Ringinglow A      | Conway <sup>5</sup>                | 400             | 20 cm above                            |
| South Pennines   | Ringinglow H      | Conway <sup>5</sup>                | 120             | 20 cm above                            |
| South Pennines   | Rishworth Moor    | Bartley <sup>16</sup>              | 190             | 10 cm above                            |
| Lancashire       | Deep Clough       | Tallis and McGuire <sup>21</sup>   | 90              | 2 cm below                             |
| North York Moors | Lady Bridge Slack | Simmons <sup>22</sup>              | 310             | 15 cm above                            |
| Lake District    | Red Tarn Moss     | Pennington <sup>23</sup>           | 150             | 7 cm above                             |
| North Pennines   | Coom Rigg B       | Chapman <sup>3</sup>               | 150             | 0 cm                                   |

## Conditions and causes of peat formation

Peat accumulates when the total respiratory activity of producers, consumers and decomposers fails to attain the same level as primary productivity. In fact it is the depression of decomposition rates which is most critical and this is related to waterlogging. Any environmental change which leads to increased waterlogging will therefore favour peat initiation. The three main variables which could be invoked to account for blanket peat formation are climate, soil maturation and human clearance of vegetation.

Frenzel<sup>24</sup> has argued the case for a short period of cold climate in many parts of the Northern Hemisphere between 5,400 and 5,000 years ago. This is disputed by Smith<sup>25</sup> who considers that much of Frenzel's data could equally be attributed to human activity, especially in upland areas. Lamb *et al.*<sup>26</sup> suggest that both rainfall and excess drainage water in Britain actually decreased by about 10% between 4000 and 2000 bc. Sources of evidence for climatic changes during this time are few, and often themselves of a botanical nature, for example, peat growth rates<sup>27</sup>, and such evidence is open to alternative interpretations. Iversen's<sup>28</sup> use of ivy, mistletoe and holly as climatic indicators on the Continent and its subsequent criticism by Troels Smith<sup>29</sup> on the grounds of prehistoric man's interest in these plants is a well known example of the misinterpretation of botanical evidence which is possible during this complex and confused period. It is generally agreed that Flandrian tree lines reached their maximum altitude at this time<sup>30,31</sup>, but their subsequent retreat cannot necessarily be regarded as an entirely climatic effect. Independent evidence for climatic change at the time of the onset of blanket peat formation is, therefore, not adequate to allow quantification.

Blanket peat could be regarded as the terminal stage in soil maturation for high rainfall areas, in which case its formation is essentially pedogenic. Godwin (in ref. 30) took his position, considering podsolisation in high precipitation/evaporation conditions a prerequisite for peat formation.

Dimbleby<sup>32</sup>, however, considers this unlikely because sub-peat soil profiles are frequently immature and do not display fully developed podsol characteristics. Since many blanket peats are developed over soils which, though presumably waterlogged, do not give the impression of having suffered intense leaching<sup>33</sup>, it is unlikely that podsolisation, pan formation and resultant drainage impedance is the main causative factor in peat initiation. In some Irish sites<sup>34,35</sup>, Mitchell has stressed the role of pan formation as a cause of waterlogging, consequent on prehistoric ploughing. Often the depth of the thin pan corresponds with the depth to which the plough penetrated. Human land use may, therefore, be intricately connected with soil processes before peat initiation at some sites, but this explanation cannot be accepted for the majority of upland peats in Britain, where neither ploughing nor podsolisation necessarily precede peat initiation. Nor is it likely that such soil processes, even in worsening climatic conditions, could be responsible for peat initiation in the absence of human forest clearance.

For many years circumstantial evidence has been accumulating which links prehistoric human activity with the initiation of blanket peat deposits<sup>18,35</sup>. Dimbleby<sup>37</sup> showed that forest could be converted into moorland as a result of Bronze Age forest clearance and Simmons<sup>18</sup> suggested that even Mesolithic communities could have initiated such changes in Dartmoor. Simmons<sup>38,39</sup> has attempted to construct an explanatory model which might account for these vegetational changes, concentrating particularly on the capacity and the motives of Mesolithic populations for producing them. He concludes that their ecological impact in upland Britain would have been small, but that their relationship with red deer as an important food resource would have provided them with ample motive for upland clearance. Neolithic peoples, complete with truly domesticated herbivores, must have had even stronger motives for upland forest clearance and the general use of such areas for grazing.

**Table 2** Summary of data concerning increased stream discharge following the deforestation of a watershed

| Site                    | Forest type              | Increased discharge on felling (%) | Author                           |
|-------------------------|--------------------------|------------------------------------|----------------------------------|
| South Appalachians, USA | Oak/hickory              | 15                                 | Swank and Douglas <sup>41</sup>  |
| Colorado, USA           | Aspen, mixed conifer     | 22                                 | Bates and Henry <sup>42</sup>    |
| Colorado, USA           | Lodgepole pine           | 25 (after 40% clearance)           | Hoover and Leaf <sup>43</sup>    |
| Japan                   | Mixed deciduous/conifers | 8-24                               | Nakano <sup>44</sup>             |
| New Hampshire, USA      | Maple/beech/birch        | 40                                 | Bormann and Likens <sup>45</sup> |



The vital question which must be asked, however, is what would be the hydrological consequences of forest modification, since peat initiation is most closely linked with soil hydrology. The question is particularly relevant to blanket peat formation since the topographical features of the sites involved are often water-shedding rather than receiving. Removal of a tree canopy from such a situation would increase the supply of ground water by reducing the transpiration demand of the vegetation and also by reducing the water interception of the canopy, much of which would evaporate once more without reaching the ground. The considerable body of experimental data now available permits the estimation of water gains resulting from forest clearance. Table 2 is a summary of data derived from experimental deforestation of watersheds bearing a variety of forest types.

There are many problems associated with the interpretation of such figures and extrapolation from them to other situations<sup>46</sup>. In particular one should be mistrustful of data from watersheds where there has been inadequate calibration before felling; one must also consider the forest types involved, since coniferous trees intercept more water than deciduous ones<sup>40</sup>; and one must take into account the climatic conditions in which the data were obtained, particularly those factors influencing evaporation. The figures obtained by Swank and Douglas are perhaps the most acceptable in terms of rigorous calibration, but in the context of a more oceanic Britain during Neolithic times, with lower evaporation rates, a figure of 10% increased discharge following clearance would be a more reasonable estimate. Studies of soil water under different vegetation types<sup>47</sup> and direct studies of interception losses in forest stands<sup>48</sup> in Hampshire, England, confirm that 10–20% more water would be available on removal of a forest canopy.

Clearcutting of forest in Finland has been reported as causing a rise in water table of 38 cm (ref. 49), and thinning of a beech forest in Denmark has resulted in the death of remaining trees by waterlogging as a consequence of the higher water table produced<sup>50</sup>. It is this kind of effect which leads to the use of fire as a means of watershed management in the USA<sup>51,52</sup>. There are even some situations in which the clearance of a catchment can be directly correlated with peat development in the areas receiving runoff. For example, Skarzynska<sup>53</sup> has described such a situation in Poland where settlements of the Luzyc culture (Iron Age) in low lying areas were literally swamped by peat-forming plant communities as a consequence of the deforestation of the surrounding catchment. In more recent times, alder/spruce woodlands in Czechoslovakia have been converted to waterlogged meadows following clearance<sup>54</sup>.

Having established that human clearance of woodland (by felling or fire) or even woodland thinning (by selective felling or free grazing by sheep or goats<sup>55</sup>) could modify hydrological regions very substantially, one must consider which prehistoric cultures may have been responsible for peat initiation in different parts of Britain. In some areas the influence of Mesolithic man in the uplands was considerable and Simmons<sup>56</sup> has implicated these cultures in his explanation of habitat changes on Dartmoor. Pennington<sup>57</sup> has described evidence for increased runoff in parts of the Lake District in late Mesolithic times and also for the development of blanket peat over mor humus and mineral soils during Neolithic times. Many radio-carbon dates for the early Neolithic are older than 5,000 yr b.p. (ref. 57) and give weight to the argument that many of the deeper, high-level blanket peats of western and northern Britain could have been initiated as a result of the activities of these peoples. Such activity may have involved no more than the free grazing of their stock coupled with occasional firing. In Ireland, the

extensive work carried out by Goddard<sup>58</sup> and Smith<sup>59</sup> suggests that, although some high altitude blanket peats began their formation during Neolithic times, the majority of peats studied were initiated in Beaker and Bronze Age times. Shallower and lower altitude peats on Exmoor<sup>14</sup> began to form during the Iron Age and during later times depending on local conditions of topography, climate and human land use, with the last-mentioned often acting in a critical manner to effect the crossing of the hydrological threshold for peat formation.

Three main conclusions emerge from these data. In the first place, prehistoric man was theoretically capable of influencing catchment hydrology in ways which would create effects of magnitude similar to those derived from major climatic changes. Second, circumstantial evidence from the dates of blanket peat initiation and from pollen assemblages at the base of these deposits, indicates man's involvement. Regional, topographical and altitudinal metachrony is to be expected. This reflects the differing intensities of man's activities experienced in various areas and also the intensity of activity required for peat initiation at different sites. Finally, the soil changes associated with peat initiation are most satisfactorily explained as a consequence rather than a cause of the demise of forest and the development of peat.

- 1 Taylor, J. A., and Tucker, R. B. *Proc. third. Int. Peat. Congr. Quebec.*, 163–173 (1968).
- 2 Moore, P. D., and Chater, E. H., *New Phytol.*, **68**, 183–195 (1969).
- 3 Chapman, S. B., *J. Ecol.*, **52**, 299–313 (1964).
- 4 Moore, P. D., *Proc. fourth Int. Peat Congr. Helsinki*, **1**, 89–100 (1972).
- 5 Conway, V. M., *J. Ecol.*, **34**, 149–181 (1947).
- 6 Conway, V. M., *J. Ecol.*, **42**, 117–147 (1954).
- 7 Tallis, J. H., *J. Ecol.*, **52**, 333–344 (1964).
- 8 Tallis, J. H., and Switsur, V. R., *J. Ecol.*, **61**, 743–751 (1973).
- 9 Smith, A. G., and Pilcher, J. R., *New Phytol.*, **72**, 903–914 (1973).
- 10 Godwin, H., *New Phytol.*, **39**, 370–400 (1940).
- 11 Godwin, H., *The History of the British Flora* (Cambridge University Press, London, 1956).
- 12 Smith, A. G., *Proc. Linn. Soc. Lond.*, **172**, 38–49 (1961).
- 13 Watts, W. A., *Proc. Linn. Soc. Lond.*, **172**, 81 (1961).
- 14 Merryfield, D. L., and Moore, P. D., *Nature*, **250**, 439–441 (1974).
- 15 Durno, S. E., and McVean, D. N., *New Phytol.*, **58**, 228–236 (1959).
- 16 Bartley, D. D., *New Phytol.*, **74**, 375–381 (1975).
- 17 Moore, P. D., *Nature*, **241**, 350–353 (1973).
- 18 Simmons, I. G., *New Phytol.*, **63**, 165–180 (1964).
- 19 Moore, P. D., *Nature*, **217**, 1006–1009 (1968).
- 20 Hicks, S. P., *New Phytol.*, **70**, 647–667 (1971).
- 21 Tallis, J. H., and McGuire, J., *J. Ecol.*, **60**, 721–737 (1972).
- 22 Simmons, I. G., *New Phytol.*, **68**, 807–827 (1969).
- 23 Pennington, W., *Proc. R. Soc., B161*, 310–323 (1965).
- 24 Frenzel, B., in *World Climate from 8,000–0 B.C.* (edit. by Sawyer, J. S.), 99–123 (Royal Meteorological Society, London, 1966).
- 25 Smith, A. G., in *Studies in the Vegetational History of the British Isles* (edit. by Walker, D., and West, R. G.), 81–96 (Cambridge University, London, 1970).
- 26 Lamb, H. H., Lewis, R. W. P., and Woodroffe, A., in *World Climate from 8,000–0 B.C.* (edit. by Sawyer, J. S.), 174–217 (Royal Meteorological Society, London, 1966).
- 27 Lamb, H. H., *Phil. Trans. R. Soc., A276*, 195–230 (1974).
- 28 Iversen, J., *Geol. Foren. Forhandl.*, **65**, H.3 (1944).
- 29 Troels-Smith, J., *Dann. Geol. Unders.*, **IV R. 4**, No. 4, (1950).
- 30 Lamb, H. H., *Q. Jl R. Met. Soc.*, **90**, 382–394 (1964).
- 31 Pears, N. V., *Trans. Bot. Soc. Edinb.*, **40**, 536–544 (1969).
- 32 Dimbleby, G. W., *Proc. R. Soc., B161*, 355–362 (1965).
- 33 Crampton, C. B., *Scott. Geogr. Mag.*, **82**, 46–52 (1966).
- 34 Case, H. J., Dimbleby, G. W., Mitchell, G. F., Morrison, M. E. S., and Proudfoot, V. B., *J. R. Soc. Ant. Irel.*, **99**, 39–53 (1969).
- 35 Mitchell, G. F., *Proc. 24th. Int. Geol. Congr. Montreal, Symp. 1.*, 59–68 (1972).
- 36 Watson, J. W., *Scott. Geogr. Mag.*, **55**, 148–161 (1939).
- 37 Dimbleby, G. W., *New Phytol.*, **51**, 349–354 (1952).
- 38 Simmons, I. G., in *The Domestication and Exploitation of Plants and Animals* (edit. by Ucko, P. J., and Dimbleby, G. W.), 111–119 (Duckworth, London, 1969).
- 39 Simmons, I. G., *J. Archaeol. Sci.*, **2**, 1–15 (1975).
- 40 Dimbleby, G. W., and Simmons, I. G., *J. Archaeol. Sci.*, **1**, 291–296 (1974).
- 41 Swank, W. T., and Douglas, J. E., *Science*, **185**, 857–859 (1974).
- 42 Bates, C. G., and Henry, A. J., *USA Mon. Wea. Rev. Suppl.*, **30**, 1–79 (1928).
- 43 Hoover, M. D., and Leaf, C. F., in *Forest Hydrology* (edit. by Sopper, W. E., and Lull, H. W.), 213–224 (Pergamon, Oxford, 1967).
- 44 Nakano, H., in *Forest Hydrology* (edit. by Sopper, W. E., and Lull, H. W.), 551–564 (Pergamon, Oxford, 1967).
- 45 Bormann, F. H., Likens, G. E., Fisher, D. W., and Pierce, R. S., *Science*, **159**, 882–884 (1968).
- 46 Hibbert, A. R., in *Forest Hydrology* (edit. by Sopper, W. E., and Lull, H. W.), 527–533 (Pergamon, Oxford, 1967).
- 47 Rutter, A. J., and Fourn, J., *appl. Ecol.*, **2**, 197–209 (1965).
- 48 Rutter, A. J., Morton, A. J., and Robens, P. C., *J. appl. Ecol.*, **12**, 367–380 (1975).
- 49 Heikurainen, L., in *Forest Hydrology* (edit. by Sopper, W. E., and Lull, H. W.), 345–354 (Pergamon, Oxford, 1967).
- 50 Holstener-Jorgensen, H., in *Forest Hydrology* (edit. by Sopper, W. E., and Lull, H. W.), 325–333 (Pergamon, Oxford, 1967).
- 51 Burma, G. D., *Proc. seventh Ann. Tall Timbers Fire Ecol. Conf.*, 235–243 (1967).
- 52 Zwolinski, M. J., and Ehrenreich, J. H., *Proc. seventh Tall Timbers Fire Ecol. Conf.*, 195–205 (1967).
- 53 Skarzynska, K., *Bull. de l'Acad. Polon. des Sci. Ser. Geol. et. Geogr.*, **13**, 241–7 (1965).
- 54 Rybníček, K., and Rybníčkova, E., *Folia Geobot. Phytotax., Praha*, **9**, 45–70 (1974).
- 55 Adams, S. N., *J. appl. Ecol.*, **12**, 143–152 (1975).
- 56 Pennington, W., *C.B.A. Res. Rept. No. 11*, 74–86 (1975).
- 57 Smith, I. F., in *British Prehistory: A New Outline* (edit. by Renfrew, C.), 100–136 (Duckworth, London, 1974).
- 58 Goddard, A., thesis, Queens Univ. Belfast (1971).
- 59 Smith, A. G., *C.B.A. Res. Rept. No. 11*, 65–74 (1975).

# articles

## Chandler Wobble, earthquakes, rotation, and geomagnetic changes

Frank Press & Peter Briggs

Department of Earth and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

*A pattern recognition algorithm can be applied to the seismicity of major earthquake belts, to the amplitudes of Chandler Wobble, to changes in the rotational velocity of the Earth, and to the drift of the eccentric geomagnetic dipole for the years 1901–64. The patterns which emerged suggest that all of these diverse phenomena are related.*

THE causes of the Chandler Wobble and of variations in the length of the day remain a subject of speculation. The suggestion that movements accompanying great earthquakes excite the wobble faces the difficulty that the displacements may not be large enough<sup>1,2</sup>. But large, slow deformations (over hours or days) can occur in the epicentral area before and after a major failure and when these movements are added to the seismic slip that occurs with the main shock, the combined displacements seem to be large enough to excite the Chandler Wobble<sup>3</sup>. Short term changes in the length of the day (over periods of about 2 yr) have been related to variations in zonal wind velocity<sup>4</sup> and long term changes (over periods of about 10 yr) have been attributed to motions in the fluid core<sup>5,6</sup>. Presumably, the motion of the eccentric dipole is related to fluid motion in the core, and the compensatory changes required to conserve angular momentum of the Earth show up as changes in the length of the day.

Anderson has suggested that volcanism and climatic changes may be related to all of these phenomena<sup>7</sup>. According to his hypothesis, major volcanic eruptions lead to zonal wind changes which affect the rotation of the Earth, both on a short and long term basis. Small perturbations in the velocity of rotation trigger the release of the large amount of elastic energy stored in the lithosphere because of rotational processes, setting off global seismic activity and exciting the Chandler Wobble. The main supporting argument is the apparent correlation between global seismicity, fluctuations in rotation and amplitudes of the Chandler Wobble, and the indication that short term zonal wind changes seem to affect the rotation rate.

Here we apply a method of pattern recognition (ref. 8 and I. M. Gelfand *et al.*, unpublished) to see if we can detect a pattern in these diverse phenomena.

### Pattern recognition algorithm

In a learning phase the computer is told into which of two groups— $G_1$  (26 yr) and  $G_2$  (38 yr), associated with the amplitude of the Chandler Wobble and its decay, respectively—the build-up of data are to be placed for each of the 64 years from 1901 to 1964. Chandler Wobble data smoothed by taking 6-yr running averages are used<sup>9</sup>.

For each year a series of heuristic questions is posed (Table 1) and answered yes/no, or 1/0 in binary code. The specific parameters for each question are assigned following a preliminary survey to obtain the best separation between  $G_1$  and  $G_2$ . For example, for Question 16 (Table 1), many time windows were tested but the values used (present year) gave the best discrimination in that 73% of  $G_1$  and only 10% of  $G_2$  received a yes answer.

### Characteristic traits for $G_1$ and $G_2$ years

The answer matrix consisting of the binary answers to the questions for each year (as well as a third category for unanswerable questions) is then examined to see if certain patterns emerge which characterise  $G_1$  or  $G_2$  years. A trait is a combination of answers to questions taken three at a time, two at a time or one at a time (triplet, doublet or singlet traits, respectively). For the 22 questions in Table 1 there are 13,288 possible traits. If a trait appears more than  $K_1$  times in  $G_1$  and less than  $\tilde{K}_1$  times in  $G_2$  it is identified as a characteristic trait for  $G_1$  years. Similarly constants  $K_2$  and  $\tilde{K}_2$  are used to identify characteristic traits for  $G_2$  years. For the questions cited in Table 1 the values  $K_1 = 14$ ;  $\tilde{K}_1 = 2$ ;  $K_2 = 27$ ; and  $\tilde{K}_2 = 2$  were used to obtain a few, in this case eight, independent characteristic traits for both  $G_1$  and  $G_2$ . In a sense, we have used pattern recognition as an hypothesis selector and have checked thousands of possible hypotheses against data.

In a final step or predictive phase, a vote is taken for each year, with each  $G_1$  characteristic trait assigned a positive vote and each  $G_2$  characteristic trait a negative vote. For each year, the algebraic sum of  $G_1$  and  $G_2$  characteristic traits (the 'netvote') is used in most applications to predict future events<sup>8</sup>, for example, to predict future build-ups or decays in the amplitude of the Chandler Wobble. These procedures may seem arbitrary, but pattern recognition lacks a formalism, as yet, and specific methods are justified by control experiments and other tests (I. M. Gelfand *et al.*, unpublished). The agreement between the netvote of characteristic traits and the Chandler Wobble amplitude data for the years 1901–64 is excellent and shows that once the computer has learned which are the characteristic traits, it can recognise and separate the years of build-up and decay of Chandler Wobble quite well using those traits alone.

It may, however, also be possible to discriminate between  $G_1$  and  $G_2$  years even if the questions were based on spurious data or random answers. In a control experiment we ran the entire program, including the preliminary one-dimensional survey, with spurious data obtained simply by reversing the time sequence of the actual data, that is, the substitution of 1964 for 1901 and so on, in obtaining answers to the questionnaire (Table 1). For the same  $K$  constants used with real data,



Table 1 Questions posed for pattern recognition

- 1 In the preceding 5th, 4th, or 3rd year WTAE0\*  $\geq 1.0$  EM-8† in the New Guinea–New Hebrides region? (Seismicity data from ref. 10 and L. Knopoff, unpublished.)
- 2 In the following 0th, 1st, 2nd year WTAE0  $\geq 1.0$  EM-8 in the New Guinea–New Hebrides region?
- 3 In the following 0th, 1st, 2nd, 3rd, or 4th year WTAE0  $\geq 0.5$  EM-8 in the Tonga–Kermadec region?
- 4 In the preceding 2nd, 1st, or 0th year WTAE0  $> 1.5$  EM-8 in the Kurile–Japan–Mariana region?
- 5 In the preceding 1st, 0th or following 1st year WTAE0  $> 2.0$  EM-8 in the Peru–Chile region?
- 6 In the preceding 3rd, 2nd, 1st, 0th or following 1st year WTAE0  $> 1.0$  EM-8 in central America?
- 7 In the preceding 2nd, 1st or 0th year WTAE0  $> 0.8$  EM-8 in Java?
- 8 In the following 0th, 1st, 2nd, 3rd, or 4th year WTAE0  $> 1.5$  EM-8 in the Ryukyu–Philippines region?
- 9 In the following 0th, 1st, 2nd, 3rd, or 4th year WTAE0  $> 0.5$  EM-8 in the San Andreas–Gorda Ridge region?
- 10 In the preceding 2nd, 1st, 0th, or following 1st or 2nd year WTAE0  $> 0.5$  EM-8 in China?
- 11 In the preceding 2nd, 1st, or 0th year WTAE0  $> 0.5$  EM-8 in the Aleutians?
- 12 In the preceding 1st, 0th, or following 1st year WTAE0  $> 0.5$  EM-8 along a continent–continent collision zone?
- 13 In the preceding 5th, 4th, 3rd, 2nd, 1st, 0th, or following 1st year WTAE0  $> 0.5$  EM-8 in the Caribbean?
- 14 In the preceding 1st, 0th, or following 1st year were there  $> 15.0$  EM-8 integrated over the whole Earth?
- 15 In the preceding 7th, 6th, 5th, 4th, 3rd, 2nd, or 1st year was there a change in the absolute value of Earth's rotational acceleration ( $|\dot{\omega}/\omega|$ ) of  $> 2 \times 10^{-9}$  in units  $\Delta\omega/\omega \text{ yr}^{-1}$  (ref. 11)?
- 16 In the present year was the absolute value of [the magnitude of Earth's rotational acceleration ( $|\dot{\omega}/\omega|$ )]  $< 1.0 \times 10^{-9}$  in units  $\Delta\omega/\omega \text{ yr}^{-1}$ ?
- 17 In the present year was the Earth's rotational acceleration negative?
- 18 In the present year was the length of day (l.o.d.) more than 0.5 ms longer than the mean value (corrected for secular slowdown)<sup>11</sup>?
- 19 In the following 2nd year was the speed ( $\dot{\nu}$ ) of the westward drift of the eccentric geomagnetic dipole  $> 0.25$  degrees  $\text{yr}^{-1}$  (ref. 5)?
- 20 In the following 3rd or 4th year was the acceleration ( $\ddot{\nu}$ ) of the eccentric geomagnetic dipole motion negative?
- 21 In the preceding 1st year was the second derivative of the squared amplitude of the Chandler Wobble positive?
- 22 In the preceding 5th, 4th, or 3rd year was there a major volcanic eruption on Earth (as defined in ref. 12)?

\*WTAE0 means 'was there an event of'.

†EM-8 signifies a measure of seismicity in units of equivalent magnitude 8 earthquakes, obtained by dividing the cumulative, annual seismic moment released in a region by the seismic moment for a magnitude 8 earthquake.

$G_2$  characteristic traits could not be found. Only by relaxing the  $K$  constants could we obtain a separation of  $G_1$  and  $G_2$  years as measured by the netvote, and even then more years were misidentified than was the case for real data.

Moreover, the traits based on the spurious data did not assemble into coherent hypotheses relating seismicity, Earth rotation and geomagnetic field drift as did the real data. It is, therefore, reasonable to expect that the characteristic traits found with real data may carry physical information, although we cannot prove this.

To test the relationship between earthquake activity and Chandler Wobble excitation a pattern recognition run was made using the first 14 questions only. Questions 1–13 refer to earthquakes in individual seismic belts and Question 14 is a measure of world seismicity. Table 2 shows the traits characteristic of  $G_1$  years of increasing Chandler Wobble amplitudes. Table 3 shows the characteristic traits for  $G_2$  years of declining Chandler Wobble amplitudes. The striking pattern which emerges is that 11 of the 13 seismic belts show either major earthquake activity associated with years of increasing amplitudes or no activity for years of decreasing amplitudes. The

Table 2 Characteristic traits for  $G_1$  years

| Trait | Question |   |   |   |   |   |   |   |   |    |    |    |    |     |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|----|-----|
|       | 1        | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14* |
| 1     | 1        | 1 | . | 0 | . | . | . | . | . | .  | .  | .  | .  | .   |
| 2     | 1        | 1 | . | . | . | 1 | . | . | . | .  | .  | .  | .  | .   |
| 3     | 1        | . | . | . | . | . | . | . | . | .  | 1  | .  | .  | .   |
| 4     | .        | 1 | . | . | . | . | . | . | . | .  | 1  | .  | .  | .   |
| 5     | .        | . | 1 | 0 | . | . | . | . | 0 | .  | .  | .  | .  | .   |
| 6     | .        | . | 1 | . | . | . | . | 1 | . | .  | .  | .  | .  | 0   |
| 7     | .        | . | . | 0 | 1 | . | . | . | . | .  | .  | .  | .  | .   |
| 8     | .        | . | . | 0 | . | 1 | . | . | . | .  | .  | .  | .  | .   |
| 9     | .        | . | . | . | 1 | 1 | . | . | . | .  | .  | .  | .  | .   |
| 10    | .        | . | . | . | 1 | . | 1 | . | . | .  | .  | .  | .  | .   |
| 11    | .        | . | . | . | 1 | . | . | . | . | .  | 1  | .  | .  | .   |
| 12    | .        | . | . | . | 1 | . | . | . | . | .  | .  | .  | .  | 1   |
| 13    | .        | . | . | . | . | 1 | 1 | . | . | .  | .  | .  | .  | .   |
| 14    | .        | . | . | . | . | 1 | . | 1 | . | .  | .  | .  | .  | .   |
| 15    | .        | . | . | . | . | 1 | . | . | . | .  | 1  | .  | .  | .   |
| 16    | .        | . | . | . | . | . | 1 | . | 1 | .  | .  | .  | .  | .   |
| 17    | .        | . | . | . | . | . | 1 | . | . | .  | 1  | .  | .  | .   |
| 18    | .        | . | . | . | . | . | . | 1 | . | .  | 1  | .  | .  | .   |
| 19    | .        | . | . | . | . | . | . | . | 1 | .  | .  | .  | 1  | .   |
| 20    | .        | . | . | . | . | . | . | . | . | .  | 1  | .  | .  | 1   |

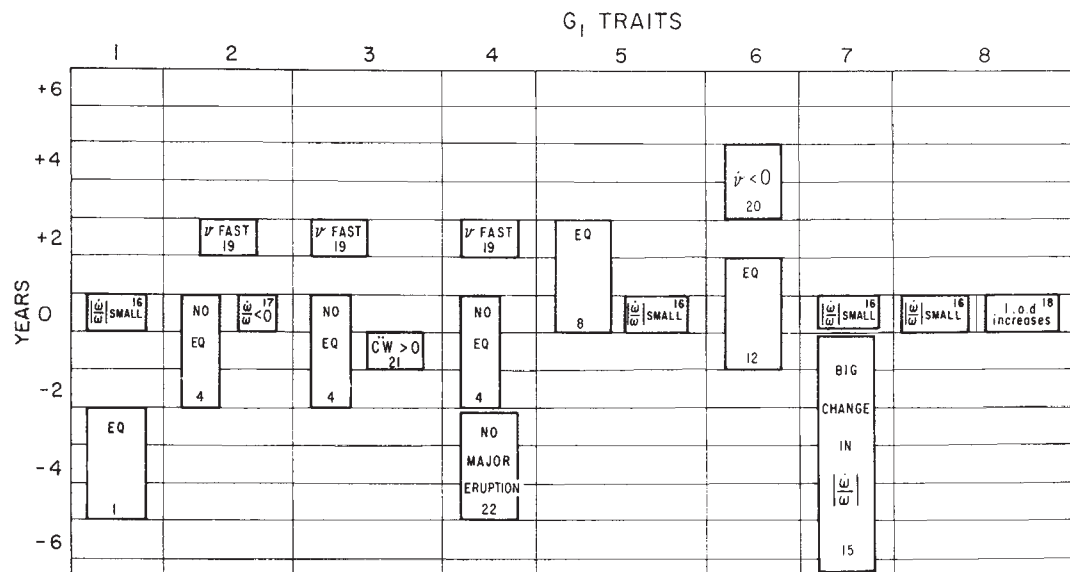
\*Columns describe activity in seismic belts (1 = yes, 0 = no); rows are combinations of answers which form individual traits.

Kurile–Japan–Mariana belt and China are the two exceptions.  $G_1$  characteristic traits occur with earthquake activity as a feature (as defined by the questions) in any one of the following seismic belts: central America, Peru–Chile, Tonga–Kermadec, New Guinea–New Hebrides and the Caribbean. Other  $G_1$  traits require earthquakes in two or three belts. World seismicity does not show up by itself as a  $G_1$  trait. These results imply a preferential excitation of the wobble by earthquakes in certain places (defined by latitude, longitude, fault and slip orientation)<sup>1</sup>. The surprising exception of the Japanese and Chinese region is a puzzle; perhaps slow deformation occurs in these belts during aseismic periods many years before or after major earthquakes. Perhaps some unknown compensatory mechanism is at work in these regions which counteracts the effect of earthquakes elsewhere. It seems that the overall pattern indicates that the excitation of the Chandler Wobble is related to earthquakes in specific places. The effect of seismic activity extends over several years before and after (mostly before) the year being tested; an expected result since the Chandler Wobble data were smoothed by taking 6-yr running averages.

The pattern recognition run for all 22 questions produced the characteristic traits shown in Figs 1 and 2. The display in

Table 3 Characteristic traits for  $G_2$  years

| Trait | Question |   |   |   |   |   |   |   |   |    |    |    |    |    |
|-------|----------|---|---|---|---|---|---|---|---|----|----|----|----|----|
|       | 1        | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 1     | .        | . | 1 | . | . | 0 | . | . | . | .  | .  | .  | 0  | .  |
| 2     | .        | . | . | 1 | 0 | . | . | . | . | .  | .  | .  | .  | .  |
| 3     | .        | . | . | 1 | . | 0 | . | . | . | .  | .  | .  | .  | .  |
| 4     | .        | . | . | 1 | . | . | . | . | . | .  | 0  | .  | .  | .  |
| 5     | .        | . | . | . | 0 | . | . | . | . | .  | .  | 0  | .  | .  |
| 6     | .        | . | . | . | 0 | . | . | . | . | .  | .  | .  | 0  | .  |
| 7     | .        | . | . | . | . | 0 | 0 | . | . | .  | .  | .  | 0  | .  |
| 8     | .        | . | . | . | . | 0 | . | . | . | .  | 0  | 0  | .  | .  |
| 9     | .        | . | . | . | . | 0 | . | . | . | .  | 0  | .  | .  | 0  |
| 10    | .        | . | . | . | . | 0 | . | . | . | .  | .  | .  | 0  | 0  |
| 11    | .        | . | . | . | . | . | . | 0 | . | .  | .  | .  | .  | 0  |
| 12    | .        | . | . | . | . | . | . | . | . | .  | 0  | .  | 0  | .  |



**Fig. 1** Display of 8  $G_1$  traits associated with years in which amplitudes of the Chandler Wobble are increasing. The component features of each trait are shown in boxes in the proper time sequence with respect to a  $G_1$  year. The number of the question (Table 1) from which the feature is derived is given in each box as an aid to the abbreviations. Eq, Earthquake; l.o.d., length of day.

these figures is organised to show both the time relationships and the way in which questions combine to form traits. Each trait is shown in a vertical column as a triplet or doublet of answers to questions; no singlets emerged as characteristic traits in this study. The vertical position of an answer reflects the time lag or advance in years with respect to the year being tested, which gave the best separation between  $G_1$  and  $G_2$  years in the preliminary one-dimensional survey mentioned earlier.

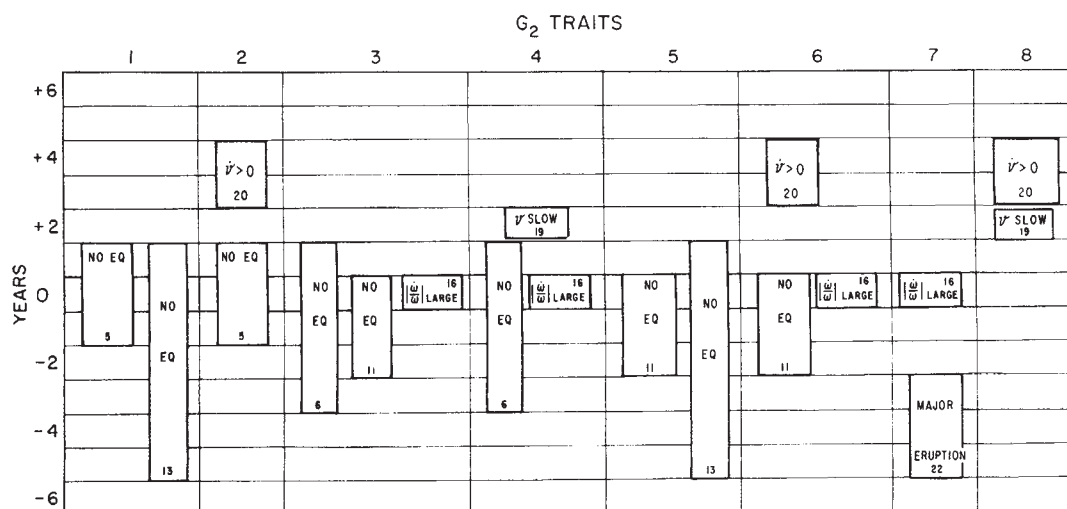
A dominant feature which emerges for  $G_1$  years is the small value of rotational acceleration (characteristic traits 1, 5, 7, 8). The Earth does not seem to undergo accelerations or decelerations in its rotational velocity,  $\omega$ , during years of increasing amplitude in the Chandler Wobble and associated increases of seismicity. Trait 7 implies a drop in the rotational acceleration during the years before a  $G_1$  year, to the small values of  $\dot{\omega}/\omega$  that go with such a year. This suggests a transition from an earlier  $G_2$  cycle when  $\dot{\omega}/\omega$  is large. Association with seismicity occurs, as discussed here, although fewer seismic belts are involved because features not involving earthquakes are now available to make up the quota of traits.

The characteristic traits for the  $G_2$  years of decaying Chandler Wobble amplitudes show an absence of seismicity (traits 1-6). The Earth's rotational acceleration is large in  $G_2$  years (traits 3, 4, 6, 7). Trait 7 identifies a period of volcanic activity ending two years before  $G_2$  years with large values of rotational acceleration. Perhaps the climatic changes induced by volcanic

dust lead to zonal wind changes which perturb the rotational velocity<sup>7</sup>; however, this trait occurs in association with  $G_2$  years when seismicity is low in 11 out of 13 earthquake belts.  $G_2$  traits 4, 6, and 8 describe a pattern in which a large change in rotational velocity occurs near a time when the westward drift is slow; this is followed by a speed-up in the westward drift of the eccentric geomagnetic dipole 3-5 yr later. Therefore, the fast but slowing drift associated with  $G_1$  years ( $G_1$  traits 2, 3, 4 and 6) may be a relic of that acceleration in drift in a preceding  $G_2$  period. The algorithm seems to have recognised the well known lag of several years between the changes in the length of the day and the motion of the eccentric dipole, which has generally been interpreted<sup>5,6</sup> as the time required for the magnetic fluctuation to diffuse through the mantle.

### Hypothesis based on pattern recognition

Although we cannot prove that the traits individually or collectively carry significant information, the patterns that emerge invite speculation. In this vein we propose the following hypothesis. Seismic activity in particular belts is associated with displacements of lithospheric plates, which are sufficient to excite the Chandler Wobble. The displacements for these particular earthquakes include as a component large preseismic and postseismic deformation. In a subsequent seismically inactive period the Chandler Wobble decays by a transfer of



**Fig. 2** Display of eight traits associated with years in which amplitudes of the Chandler Wobble are decreasing. Abbreviations as in Fig. 1.



angular momentum to the Earth's fluid core. The core motions produce compensatory changes in the rotation of the Earth, conserving angular momentum. The motions in the core also perturb the geomagnetic field, which becomes evident on the surface after several years of diffusion through the mantle.

The location of the energy sink for the Chandler Wobble is a subject of considerable speculation<sup>13</sup>. The notion that emerges from pattern recognition, that the wobble is damped by a transfer of its angular momentum to the core, should be analysed quantitatively for feasibility. Perhaps a non-spherical or bumpy core-mantle interface facilitates the transfer. Pattern recognition indicates that tectonic movements excite the wobble. The proposal that both may have a common cause cannot be ruled out, although its acceptance is unnecessary now that slow deformation and seismic slip in combination seem sufficient to excite the wobble.

This study was supported by the US Geological Survey, Department of the Interior. We are grateful to I. M. Gelfand, Sh. A. Guberman, and V. I. Keilis-Borok for making the

pattern recognition algorithm available to us. W. H. Press suggested that a spurious data test using time-reversed data was more convincing than one based on an answer matrix with random answers.

Received April 21; accepted June 5, 1975.

- <sup>1</sup> Dahlen, F. A., *Geophys. J.*, **32**, 208-217 (1973).
- <sup>2</sup> Smylie, D. E., and Mansinha, L., *Geophys. J.*, **23**, 329-354 (1971).
- <sup>3</sup> Kanamori, H., and Cipar, J. J., *Phys. Earth planet. Interiors*, **9**, 128-136 (1974).
- <sup>4</sup> Lambeck, K., and Cazenave, A., *Geophys. J.*, **32**, 79-93 (1973).
- <sup>5</sup> Kahle, A. B., Ball, R. H., and Cain, J. C., *Nature*, **223**, 165 (1969).
- <sup>6</sup> Vestine, E. H., and Kahle, A. B., *Geophys. J.*, **15**, 29-38 (1968).
- <sup>7</sup> Anderson, D. L., *Science*, **182**, 49-50 (1974).
- <sup>8</sup> Bongard, M. M., Vaincvaig, M. H., Guberman, Sh. A., Izvekova, M. L., and Smirnov, M. S., *Geologiya Geofiz. Novosibirsk*, **6**, 96-105 (1966).
- <sup>9</sup> Myerson, R. J., in *Earthquake Displacement Fields and the Rotation of the Earth*, (edit. by Mansinha, L., Smylie, D. E., and Beck, A.), 159-168 (Springer, New York, 1970).
- <sup>10</sup> Duda, S., *Tectonophysics*, **2**, 409-452 (1965).
- <sup>11</sup> Markowitz, W., in *Earthquake Displacement Fields and the Rotation of the Earth*, (edit. by Mansinha, L., Smylie, D. E., and Beck, A.), 68-81 (Springer, New York, 1970).
- <sup>12</sup> Lamb, H., *Phil. Trans. R. Soc.*, **A266**, 425-534.
- <sup>13</sup> Rochester, M. G., in *Earthquake Displacement Fields and the Rotation of the Earth*, (edit. by Mansinha, L., Smylie, D. E. and Beck, A.), 3-13 (Springer, New York, 1970).

## A-Protein gene of bacteriophage MS2

W. Fiers, R. Contreras, F. Duerinck, G. Haegmeant, J. Merregaert, W. Min Jou, A. Raeymakers, G. Volckaert & M. Ysebaert

Laboratory of Molecular Biology,

J. Van de Kerckhove, F. Nolf & M. Van Montagu

Laboratory of Histology and Genetics, State University of Ghent, Ledeganckstr. 35, B-9000 Ghent, Belgium.

*The nucleotide sequence of the A-protein gene of bacteriophage MS2 has been determined and a model for its secondary structure is proposed. Also the amino acid sequence of the A-protein has been almost completely elucidated.*

BACTERIOPHAGE MS2, and the closely related R17 and f2, contain three genes coding for the A-protein, the coat protein and the RNA polymerase subunit<sup>1,2</sup>. The first gene is located 5'-proximal<sup>3</sup>. The A-protein contains five histidine residues, whereas the coat contains none<sup>4</sup>. It has a molecular weight of 38,000-42,000 and is present in one copy per virion<sup>5-7</sup>. A-protein is required for proper encapsidation as infection with an amber A-mutant in non-permissive conditions leads to production of non-infectious particles, which have a normal shell of coat protein, but from which part of the viral RNA 'dangles' out<sup>8-10</sup>. Selective removal of A-protein by high salt treatment of the virions correlates closely with loss of viability<sup>11</sup>. Although in a normal infection the A-protein enters the host cell together with the RNA and may have a role in the early cytoplasmic events<sup>12,13</sup>, it is apparently not absolutely essential as free viral RNA is infectious in a spheroplast system<sup>14</sup>.

In R17 the A-protein gene starts with A-U-G and the nucleotide sequence of the surrounding ribosome binding region has been elucidated<sup>15</sup>. We have shown that MS2 RNA starts with a 129-nucleotide long untranslatable leader sequence<sup>16,17</sup> and that the initiation codon of the A-protein gene is G-U-G (ref. 18). The termination signal is U-A-G and in *su<sup>+</sup>* strains partial read-through occurs<sup>7</sup>. We have reported previously the last 135 nucleotides of the A-protein gene and have shown that the latter is followed by an intercistronic region 26 nucleotides long<sup>19</sup>. Some internal segments both of the

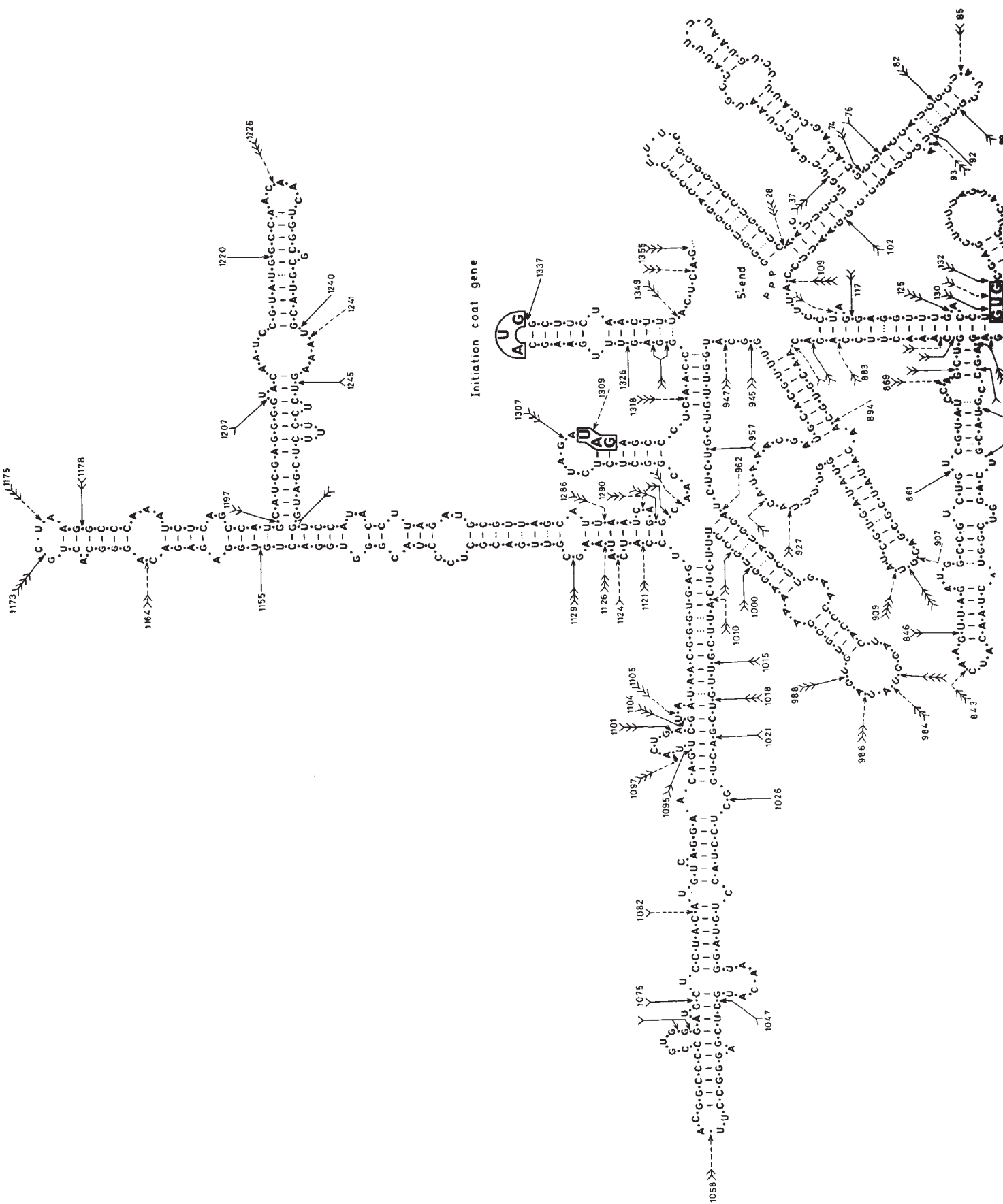
R17 and of the MS2 A-protein gene have also been published<sup>20-24</sup>, as was the carboxyl-terminal amino acid sequence of the A-protein<sup>25</sup>.

Now the complete nucleotide sequence of the A-protein gene has been determined. Also presented in this paper is a tentative model of the secondary structure. Moreover, in an independent effort the amino acid sequence of the MS2 A-protein itself has been almost completely elucidated.

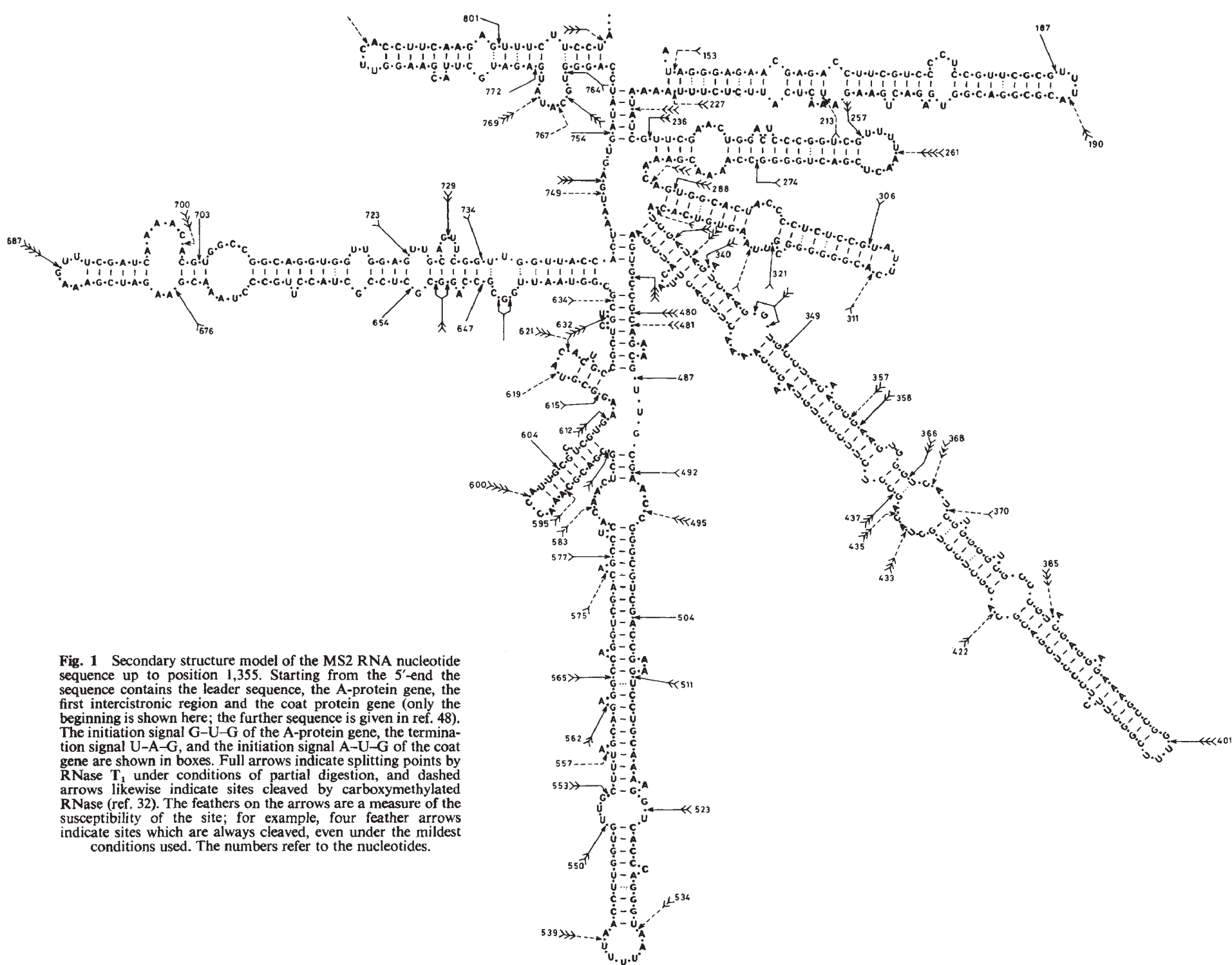
### Nucleotide sequence determination

By virtue of its specific secondary and tertiary structure highly <sup>32</sup>P-labelled MS2 RNA can be partially digested with RNase T<sub>1</sub> to yield discrete products<sup>26,27</sup>. Such a partial digest was first fractionated by electrophoresis on neutral polyacrylamide gel slabs and the resulting bands were further resolved into individual, pure fragments by two-dimensional gel electrophoresis<sup>17,28</sup>. These fragments were of chain length 25-250 and generally adequate for complete sequencing according to the methods developed by Sanger<sup>29</sup> or some modifications thereof<sup>17,24,30</sup>. Several fragments were often found to be related to each other (for example, one containing an extension of the other) and hence were derived from the same region of the RNA molecule; these related fragments are called a family. Many families could be identified by the presence of a unique T<sub>1</sub>-oligonucleotide, like A-A-C-A-G or U-A-A-A-G (occurring once in MS2 RNA).

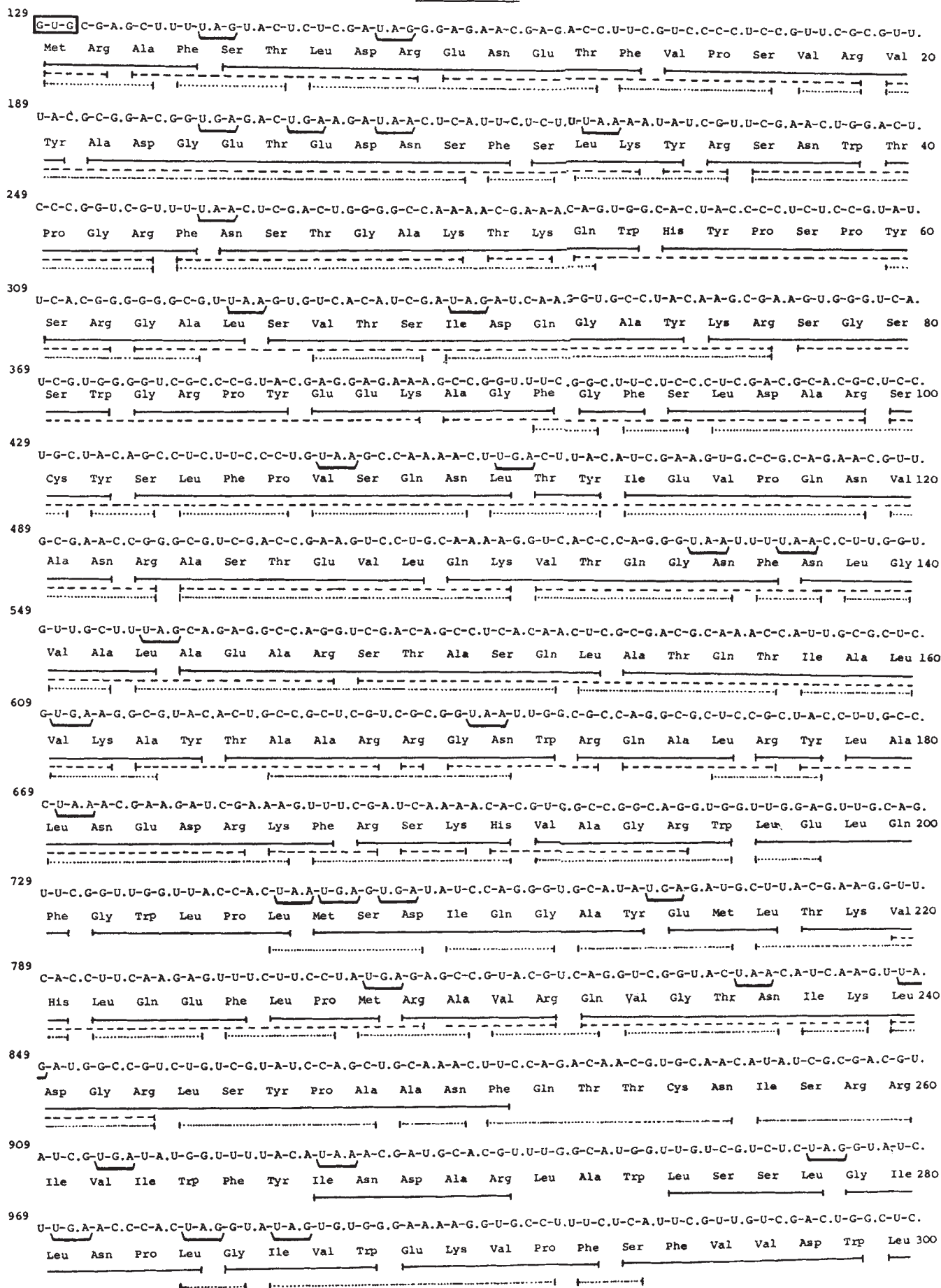
Some polypurine tracts have previously been located in the 5'-third of MS2 RNA<sup>31</sup> and on this basis some families could be assigned to the A-protein gene. Very mild T<sub>1</sub> digests, followed by separation on a 4% polyacrylamide gel, allowed the isolation of fragments in the size range of 200-1,000 nucleotides. In this







## A-PROTEIN GENE





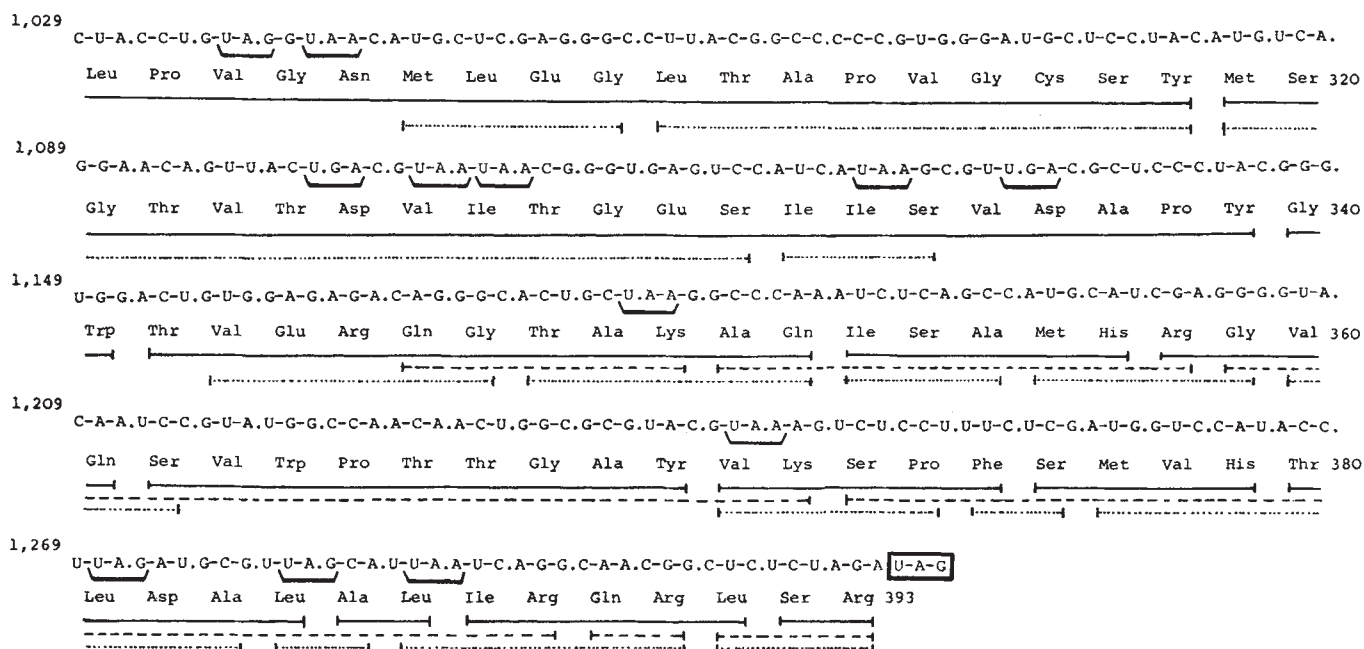


Fig. 2 Nucleotide sequence and amino acid sequence of the A-protein gene. Full lines indicate chymotrypsin peptides, dashed lines trypsin peptides and pointed lines thermolysin peptides; all these were completely sequenced. Also indicated are the out-of-phase termination signals. The number of nucleotides (from the 5'-end) are shown at the left and the number of amino acids at the right.

way all families belonging to the A-protein gene could be identified and roughly ordered. Unambiguous overlaps, however, were established by partial digestion of MS2 RNA with carboxymethylated (CM) ribonuclease<sup>32</sup>; this enzyme cleaves mainly C-A and U-A bonds. The CM partial digests of total MS2 RNA were fractionated in the same way as the T<sub>1</sub> digests. The resulting CM fragments not only provided all the required overlaps, but also allowed to sequence some regions which were represented in poor yield in the set of T<sub>1</sub> fragments.

For example the T<sub>1</sub> fragments 133-366 (positions are given in nucleotide units) and 367-632 were characterised. The former could be linked to the 5'-end<sup>16-18</sup> by virtue of the CM fragment 110-261, which a.o. contains the unique reference oligonucleotide A-G-G-A-G-G-U. Both T<sub>1</sub> fragments could then be linked by the CM fragment 339-433. Another CM fragment, 601-700 overlapped the second and the third T<sub>1</sub> family and so on. The detailed derivation of the nucleotide sequence will be reported elsewhere.

The nucleotide sequence of the A-protein gene, together with the preceding 5'-leader sequence and the succeeding link to the coat protein gene is presented in Fig. 1 in the form of a secondary structure model. The latter is partly based on experimental evidence, such as segments which fractionate together unless denaturing conditions are used, and susceptibility of sites to cleavage by the largely single-strand specific T<sub>1</sub> or CM RNases. Furthermore the thermodynamic stability of alternative configurations can be estimated<sup>33</sup> and on this basis the stability of the secondary structure model can be maximised. These estimates, however, involve many uncertainties. Moreover, the structure is certainly further moulded by tertiary interactions, as have been identified in tRNA (ref. 34). For these reasons the model shown in Fig. 1 should be regarded as tentative.

### Amino acid sequence

Pure MS2 A-protein was obtained by isolation of the A-protein-RNA complex according to Osborn *et al.*<sup>35</sup>, phenolisation, chromatography on a Sephadex G-200 column in the presence of 1% sodium dodecyl sulphate (SDS) and further separation by preparative polyacrylamide electrophoresis in the presence

of SDS. Starting from 5 g of virus 25 mg A-protein was obtained. The carboxymethylated A-protein was digested with appropriate proteolytic enzymes. The soluble peptides were fractionated by gel filtration on Sephadex G-25 in 5% formic acid followed by a three-dimensional combination of electrophoresis and paper chromatography<sup>36</sup>. Peptides were detected with fluorescamine and characterised by the micro dansyl-Edman procedure<sup>37</sup>. Several "insoluble" peptides were labelled with fluorescamine<sup>38</sup> and purified by electrophoresis in the presence of SDS on 17.5% polyacrylamide gel. The results of the amino acid sequence determination are summarised in Fig. 2. Further methodological details will be published elsewhere. Note that the first seven amino acids and the last three were determined on the complete polypeptide.

Except for a few gaps and missing overlaps, the amino acid sequence can be reconstructed without reference to the nucleotide sequence. It is in total agreement with the amino acid sequence derived from the nucleotide sequence on the basis of the known universal genetic code. Occasional apparent discrepancies were encountered, for example a threonine in position 245. But analysis of the same virus stock as used for nucleotide sequence analysis revealed the presence of serine at this position.

### Biological implications

As previously discussed<sup>18</sup>, the MS2 A-protein gene starts with G-U-G. Although more than 10 ribosome binding regions have been identified so far in phage and bacterial messengers<sup>2,39</sup> all other normal initiation codons are A-U-G (G-U-G, however, seems to be a functional start codon in translational reinitiation<sup>40</sup>). The initiation site of the R17 A-protein gene contains also A-U-G (ref. 15), in spite of the fact that the sequence of the region is otherwise identical to that of MS2 and that the level of gene expression is approximately the same in the two phages. The starting methionine residue is not removed after biosynthesis; the same is true for the analogous sequence Met-Arg-Val... of the UDP galactose 4-epimerase<sup>41</sup>. The first eight amino acids of R17 have previously been determined<sup>42</sup>; they are identical except for a Thr→Ala change.

The code words used in the A-protein message are summarised in Fig. 3. Except for U-G-U (which does occur in the coat protein gene) all sense codons are found in the A-protein gene. It is quite possible that protein synthesis is also regulated at the level of chain elongation rate and this could be due to modulating codons which would locally brake the speed of the ribosomes. Therefore the latter type of codons would be avoided in a gene expressed at high frequency such as the coat protein gene. Candidates for such a modulating function are a.o. A-U-A, A-G-A and A-G-G. One may also note that for

|   | U                        | C                        | A                                 | G                         |                  |
|---|--------------------------|--------------------------|-----------------------------------|---------------------------|------------------|
| U | Phe { 6<br>10<br>8<br>⑥  | Ser { 5<br>6<br>8<br>10  | Tyr { ④<br>12<br>Ochre<br>Amber o | Cys { 3<br>Opal<br>Trp 12 | U<br>C<br>A<br>G |
| C | Leu { 6<br>9<br>5<br>②   | Pro { 5<br>5<br>4<br>3   | His { ②<br>③<br>9<br>9            | Arg { 7<br>6<br>⑥<br>③    | U<br>C<br>A<br>G |
| A | Ile { 1<br>8<br>⑦        | Thr { 11<br>5<br>⑤<br>6  | Asn { 2<br>15<br>5<br>9           | Ser { ④<br>3<br>③<br>④    | U<br>C<br>A<br>G |
| G | Val { 8<br>7<br>7<br>⑦+8 | Ala { 6<br>12<br>7<br>10 | Asp { 8<br>5<br>5<br>12           | Gly { 15<br>6<br>2<br>5   | U<br>C<br>A<br>G |

Fig. 3 Code words used in the A-protein gene. The frequency of occurrence of each code word is given. ⊙, Initiation codon; ○, termination codon. Numbers encircled indicate codons not used in the coat gene; note that the coat protein does not contain histidine.

some amino acids (Ile, Asn, Gly) one degenerate codon is much preferred over the other (Fig. 3). The secondary folding of the viral RNA is certainly very important both from a functional and from a structural point of view. Yet it is not evident whether degeneracy of the code plays a role in this respect; in the model of the A-protein gene (Fig. 1), 67.4% of all the nucleotides are involved in secondary interactions, while for third letters this amounts to 68.4%, that is only slightly higher. The occurrence of termination signals in the two illegitimate reading frames (Fig. 2) is about as expected from a random distribution.

The ribosome binding region of the A-protein gene is only accessible after partial degradation and loss of three-dimensional folding of the viral RNA (ref. 43). It has been proposed that this gene is expressed on chains *statu nascendi* and turned off on subsequent folding of the viral RNA<sup>2,44,45</sup>. We have experimental evidence which supports this hypothesis. This model of auto-control at a translational level can be explained on the basis of the secondary structure (Fig. 1). When nearly two-thirds of the A-gene has been synthesised, a region appears (874-885) which interacts with part of the ribosome binding region and in this way closes off access to the latter. Further information is required, however, to prove this structure-function relationship.

Sixty to seventy per cent of all MS2 amber mutants are affected in the A-protein gene<sup>46</sup>. There are 18 glutamine residues in the A-protein, of which nine are coded by C-A-G (most amber mutations are C-A-G→U-A-G transitions). Yet more than one-third of the A-protein amber mutations occur at a

single site, resulting in non-permissive conditions in a polypeptide 88% of full length<sup>47</sup>. This mutational hot spot corresponds to the C-A-G at position 1,164-1,166 (Fig. 1); obviously it is rather exposed and therefore easily accessible to mutagenic reagents. A similar conclusion has previously been made for the mutation-prone C-A-G codons in the coat gene<sup>48</sup>.

Compared with the coat protein the A-protein has a higher leucine to isoleucine ratio (factor 2.6), a higher tryptophan content (factor 2.0) and in particular a higher arginine content (factor 2.4). The abundant arginine residues (for example, in regions 168-177 185-195 and 388-393) may account for the strong binding to the RNA. Indeed the interactions between the A-protein and the other components of the virion are salt sensitive<sup>11</sup>. Also some clustering of hydrophobic amino acids is observed (such as the regions 196-206, 261-267 and 272-303) and this may explain the general stickiness of the A-protein. It is conceivable that during the formation of the capsid a single coat protein molecule (out of the 180) is replaced by an A-protein molecule. There are no regions with extensive sequence homology in these two molecules however.

We thank R. Thijs, M. Bensch, A. Lenaerts and J. Gielen for technical assistance and R. De Wachter and D. Iserentant for advice. R.C., G.H. and J.V. thank the Belgian NFWO for fellowships. This research was supported by a grant from the Belgian FKFO.

Received May 28; accepted June 16, 1975.

- 1 Fiers, W., in *Handbook of Genetics* (edit. by King, R. C.), 1, 271-294 (1974).
- 2 Weissmann, C., Billeter, M. A., Goodman, H. M., Hindley, J., and Weber, H., *A. Rev. Biochem.*, **42**, 303-328 (1973).
- 3 Jeppesen, P. G. N., Argetsinger-Steitz, J., Gesteland, R. F., and Spahr, P. F., *Nature*, **226**, 230-237 (1970).
- 4 Argetsinger-Steitz, J., *J. molec. Biol.*, **33**, 937-945 (1968).
- 5 Nathans, D., Oeschger, M. P., Eggen, K., and Shimura, Y., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1844-1851 (1966).
- 6 Argetsinger-Steitz, J., *J. molec. Biol.*, **33**, 923-936 (1968).
- 7 Remaut, E., and Fiers, W., *J. molec. Biol.*, **71**, 243-261 (1972).
- 8 Lodish, H. F., Horiuchi, K., and Zinder, N. D., *Virology*, **27**, 139-155 (1965).
- 9 Argetsinger, J. E., and Gussin, G. N., *J. molec. Biol.*, **21**, 421-434 (1966).
- 10 Heisenberg, M., *Biochem. biophys. Res. Commun.*, **27**, 131-137 (1967).
- 11 Verbraeken, E., and Fiers, W., *FEBS Lett.*, **28**, 89-92 (1972).
- 12 Kozak, M., and Nathans, D., *Nature*, **234**, 209-211 (1971).
- 13 Krahn, P. M., O'Callaghan, R. J., and Paranchych, W., *Virology*, **47**, 628-627 (1972).
- 14 Davis, J. E., Strauss, J. H., and Sinsheimer, R. L., *Science*, **134**, 1427 (1961).
- 15 Argetsinger-Steitz, J., *Nature*, **224**, 957-964 (1969).
- 16 De Wachter, R., Vandenberghe, A., Merregaert, J., Contreras, R., and Fiers, W., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 585-589 (1971).
- 17 De Wachter, R., Merregaert, J., Vandenberghe, A., Contreras, R., and Fiers, W., *Eur. J. Biochem.*, **22**, 400-414 (1971).
- 18 Volckaert, G., and Fiers, W., *FEBS Lett.*, **35**, 91-96 (1973).
- 19 Contreras, R., Ysebaert, M., Min Jou, W., and Fiers, W., *Nature new Biol.*, **241**, 99-101 (1973).
- 20 Rensing, U. F. E., *Biochem. J.*, **131**, 593-604 (1973).
- 21 Rensing, U. F. E., and Coulson, A., *Biochem. J.*, **131**, 605-610 (1973).
- 22 Rensing, U. F. E., and Schoenmakers, J. G. G., *Eur. J. Biochem.*, **33**, 8-18 (1973).
- 23 Rensing, U. F. E., Coulson, A., and Schoenmakers, J. G. G., *Eur. J. Biochem.*, **41**, 431-438 (1974).
- 24 Haegeman, G., and Fiers, W., *Eur. J. Biochem.*, **36**, 135-143 (1973).
- 25 Vandekerckhove, J., Nolf, F., and Van Montagu, M., *Nature new Biol.*, **241**, 102 (1973).
- 26 Min Jou, W., Hindley, J., and Fiers, W., *Archs intern. Physiol. Biochim.*, **76**, 194-195 (1968).
- 27 Adams, J. M., Jeppesen, P. G. N., Sanger, F., and Barrell, B. G., *Nature*, **223**, 1009-1014 (1969).
- 28 De Wachter, R., and Fiers, W., *Analyt. Biochem.*, **18**, 351-374 (1967).
- 29 Sanger, F., and Brownlee, G., *Methods in Enzymology*, **12A** (edit. by Colowick, S. P., and Kaplan, N. O.), 361-381 (Academic, New York and London, 1967).
- 30 Contreras, R., and Fiers, W., *Analyt. Biochem.* (in the press).
- 31 Min Jou, W., Fiers, W., Goodman, H., and Spahr, P., *J. molec. Biol.*, **42**, 143-146 (1969).
- 32 Contreras, R., and Fiers, W., *FEBS Lett.*, **16**, 281-283 (1971).
- 33 Tinoco, I., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M., and Gralla, J., *Nature new Biol.*, **246**, 40-41 (1973).
- 34 Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., and Klug, A., *Nature*, **250**, 546-551 (1974).
- 35 Osborn, M., Weiner, A. M., and Weber, K., *Eur. J. Biochem.*, **17**, 63-67 (1970).
- 36 Milstein, C., *Biochem. J.*, **110**, 652-654 (1968).
- 37 Bruton, C. J., and Hartley, B. S., *J. molec. Biol.*, **52**, 165-173 (1970).
- 38 Vandekerckhove, J., and Van Montagu, M., *Eur. J. Biochem.*, **44**, 279-288 (1974).
- 39 Maizels, N., *Nature*, **249**, 647-649 (1974).
- 40 Fries, J. G., Weber, K., and Miller, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 667-670 (1974).
- 41 Musso, R. E., De Crombrughe, B., Pastan, I., Sklar, J., Yot, P., and Weissman, S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4940-4944 (1974).
- 42 Weiner, A. M., Platt, J., and Weber, K., *J. Biol. Chem.*, **247**, 3242-3251 (1972).
- 43 Argetsinger-Steitz, J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2605-2609 (1973).
- 44 Robertson, H. D., and Lodish, H. F., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 710-716 (1970).
- 45 Kozak, M., and Nathans, D., *Bact. Rev.*, **36**, 109-134 (1972).
- 46 Fiers, W., et al., *Cold Spring Harb. Symp. quant. Biol.*, **34**, 697-706 (1969).
- 47 Vandamme, E., Remaut, E., Van Montagu, M., and Fiers, W., *Molec. gen. Genet.*, **117**, 219-228 (1972).
- 48 Min Jou, W., Haegeman, G., Ysebaert, M., and Fiers, W., *Nature*, **237**, 82-88 (1972).



# Late Miocene sediments and fossils from the Northern Kenya Rift Valley

M. Pickford

Department of Geology, Queen Mary College, University of London, UK

*The Lukeino Formation occurs within a long sequence of Neogene sedimentary units that are soundly calibrated by K-Ar dates. It has yielded an abundant and varied fauna and flora, and contains a wealth of information on palaeoenvironments and sedimentary processes occurring during its deposition. Previously unpublished vertebrate and invertebrate assemblages, about 6.5 Myr old, are recorded here, and the stratigraphic and sedimentary framework of the formation is established.*

THE Lukeino Formation was mapped during 1973 as part of the programme dealing with all the sedimentary units in the Baringo area, Kenya, based at Bedford College, London, under Professor W. W. Bishop<sup>1</sup>. This work has

resulted in the elucidation of the geographic extent, stratigraphic succession, facies changes, palaeoenvironments and palaeogeomorphological setting of the basin. During the survey many vertebrate and invertebrate fossils were collected including the crown of a hominid molar. Mapping has also shown that fossils collected previously from the Kaperyon area actually came from the Lukeino Formation. The Lukeino Formation was examined cursorily by Bishop *et al.*<sup>1</sup> in 1971, and this paper, apart from recording numerous new faunal elements, discusses in detail the sediments themselves.

## Lukeino Formation

The Lukeino Formation (Figs 1–3) is composed of up to 130 m of primary and reworked volcanic rock. The sediments were deposited in a basin flooded by the Kabarnet Trachyte Formation<sup>2</sup> and are overlain by the Kaparaina Basalt Formation<sup>3</sup>. Three sills have intruded the formation.

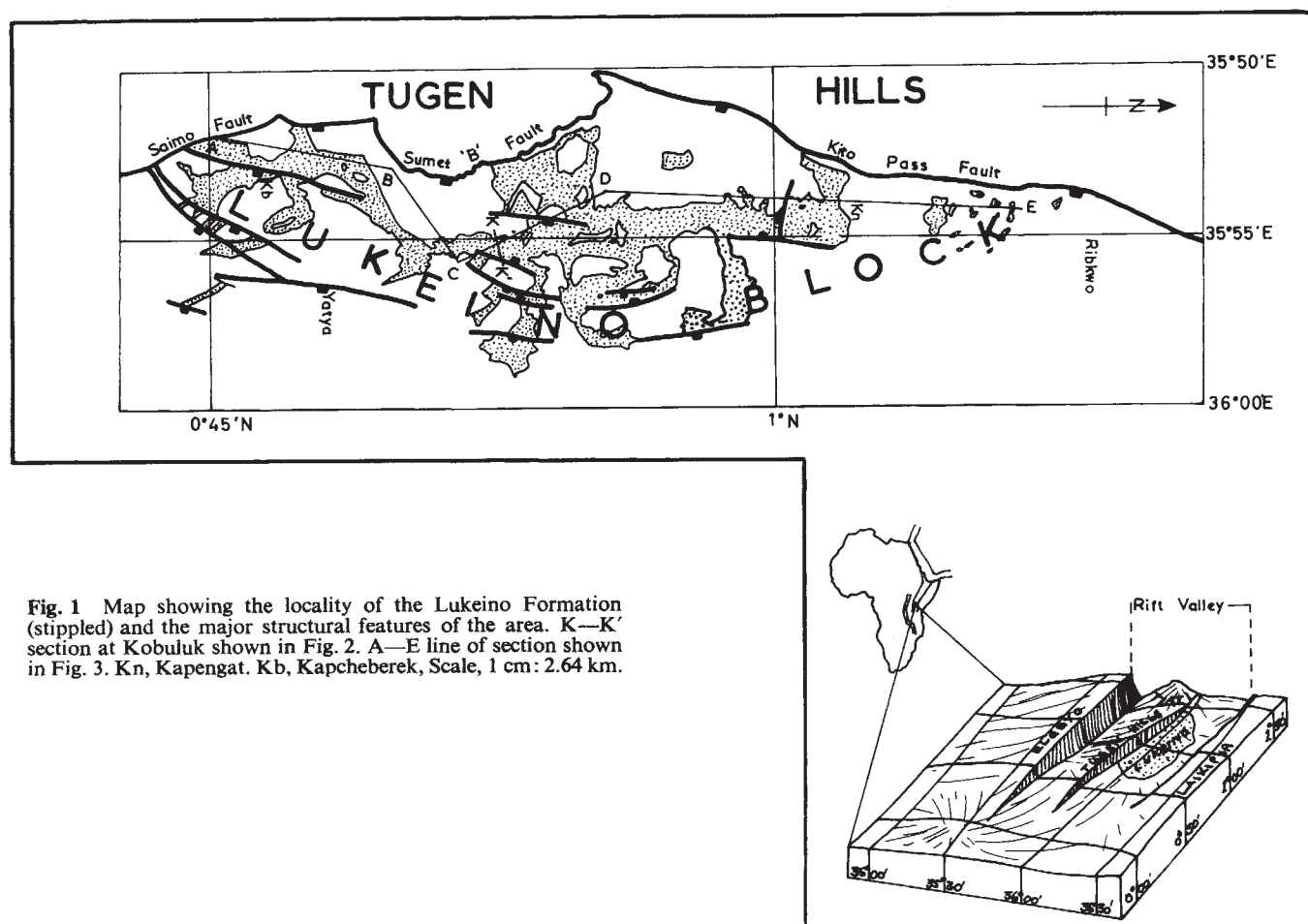


Fig. 1 Map showing the locality of the Lukeino Formation (stippled) and the major structural features of the area. K—K' section at Kobuluk shown in Fig. 2. A—E line of section shown in Fig. 3. Kn, Kapengat. Kb, Kapcheberek. Scale, 1 cm: 2.64 km.

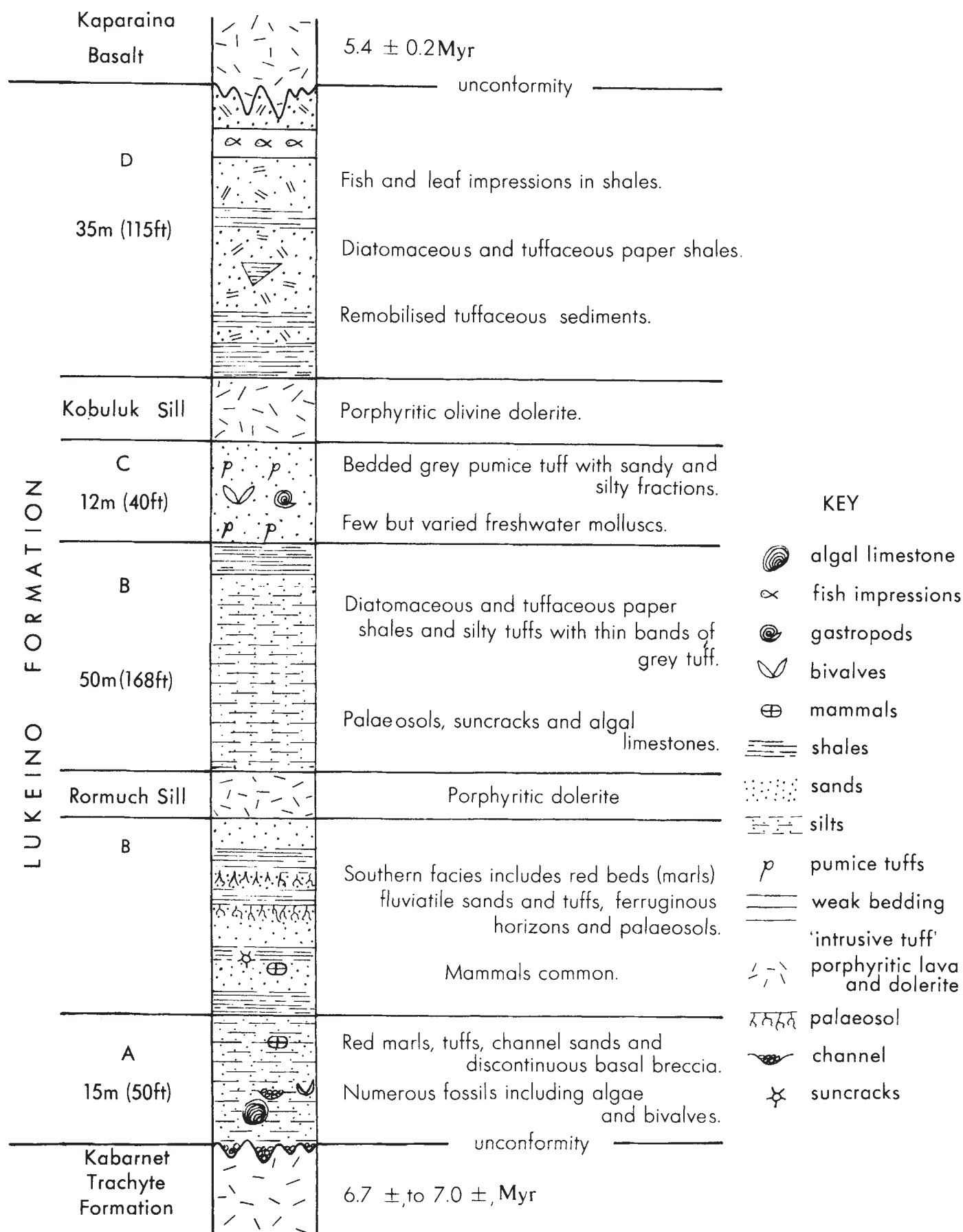


Fig. 2 Type section of Lukeino Formation at Kobuluk.



The Lukeino unit is given formational status because it is clearly not part of the Kaparaina Basalt Formation in which it was originally included<sup>1</sup>. The maximum observed thickness occurs at Kobuluk (section K-K', Fig. 1) north of Yatya (0°52'30"N, 35°54'30"E) where 130 m of sediments are seen in an incomplete but continuous section. The base of the formation is not exposed in this area and the top was eroded before extrusion of the lowest local flow of Kaparaina Basalt. Extrapolation along strike suggests, however, that little is missing from the Kobuluk section. Poor exposure over much of the remaining outcrop of the Lukeino Formation has severely hampered stratigraphic study but the general relationships have been deciphered. Four members are recognised in the formation (A to D, Fig. 2).

A (15 m) is composed predominantly of red sediment but contains pale orange and yellow tuffaceous horizons. Occasional sand and grit-filled channels occur from which various fossils have been obtained including a hominid molar. Much of the epiclastic material is derived from ferruginous weathering products of the Kabarnet Trachyte. Varying amounts of pyroclastic material, possibly from contemporary volcanic sources as well as from the underlying Mpesida Beds<sup>1</sup>, are mixed with the derived weathering products. The red beds seem to contain marginal lacustrine deposits as indicated by the presence of algal mats and lacustrine bivalves (including complete specimens with valves in the closed position).

The central and northern parts of B (50 m) consist of diatomaceous and pale cream silty tuffs, giving way southwards to red beds similar to those of A.

Well-bedded, grey, sandy and silty lapilli tuffs form a widespread Member C (12 m). They may be an extension northwards of the Riwo Beds of Martyn<sup>4</sup> which outcrop east of Kabarnet, about 35 km south-west of Yatya. The tuffs in the northern exposures contain a variety of lacustrine molluscs, while in the south they have yielded a few mammal bones. They are in general poorly fossiliferous.

Diatomaceous and silty tuffs comprise the major portion of Member D (35 m). Minor amounts of pumice tuff occur in the sequence except in the Kobuluk River, 2 km north of Kobuluk section, where a very thick succession of tuffs, agglomerates and breccias indicates proximity to a volcanic centre (Fig. 4). Away from the centre there are widespread fine-grained diatomaceous silts and tuffs which in places are exceptionally rich in fish impressions.

In the central parts of the basin, an extensive silty tuff outcrops in the diatomaceous sequence. The remarkable aspect of this unit is its 'intrusive' appearance. Its upper surface has many structures usually associated with igneous intrusions, such as cross cutting, stoping and distortion of bedding, but no baking was seen. This unit extends at least 12 km from north to south. Close examination shows that it is made up of small fragments of pre-existing sediments, sometimes well-laminated and it seems to represent a remobilised tuff. The intrusive nature of the contacts

possibly results from slumping of an unconsolidated stratum, accompanied by rupturing of overlying and underlying laminated diatomaceous tuffs.

## Sills

Three sills have intruded the Lukeino Formation, two of which were emplaced before the deposition of Member D. The lowermost of these (The Rormuch Sill) is a coarse porphyritic dolerite with a fine-grained matrix. Honey-coloured feldspar phenocrysts up to 2 cm long are scattered throughout the sill, but become smaller northwards. Intrusive contacts are displayed in several outcrops. The second sill (the Kobuluk Sill) is finer grained than the Rormuch Sill, and contains olivine phenocrysts up to 2 mm long set in a fine-grained grey matrix. The upper parts of this unit are often vesicular. The upper surface of this sill is often flow-banded and possesses pressure ridges, reminiscent of ropy lava, but its intrusive character is indicated in several outcrops. Fragments of both sills are found in the agglomerates of the Kobuluk River volcanic centre, which must therefore postdate the emplacement of the sills.

The third sill (the Cheseton Sill) has intruded sediments overlying tuffs and agglomerates of the Kobuluk River volcanic centre. It is very fine grained with occasional feldspar phenocrysts and indeed resembles a basalt flow. Its intrusive nature is indicated by a glassy upper surface, baked overlying sediments and the observation that it changes horizon.

## Post-Lukeino times

Relative uplift of the Lukeino area after the deposition of Member D resulted in erosion of the formation, and in places valleys were cut through the sediments to the underlying Kabarnet Trachyte. The eroding landscape was eventually capped by basalts of the Kaparaina Formation, although in the north the basalts are now absent and the Lukeino Formation is overlain unconformably by sediments of the Chemeron Formation (Fig. 3 and ref. 5). The northern part of the Lukeino Block may not have been covered by Kaparaina Basalt, a suggestion prompted by thinning of the Basalts and a decrease in the number of flows northwards.

## Palaeogeomorphology and palaeoenvironments

Fig. 4 shows the palaeoenvironments and facies distribution in the Lukeino Formation. The Lukeino basin was initiated as a result of tectonic activity along the Kito Pass, Sumet 'B' and Saimo faults (Fig. 1) which formed the western boundary against which the sediments were ponded. The large mountain mass of Tiati volcano to the north was the probable northern bounding feature (Fig. 1). The Lukeino sediments are still relatively thick where they disappear under the Ribkwo Trachyte in the north of the area. To the south the sediments are obscured under an extensive pile of Kaparaina Basalt. To the east the sediments are downfaulted and covered by up to 280 m of Kaparaina

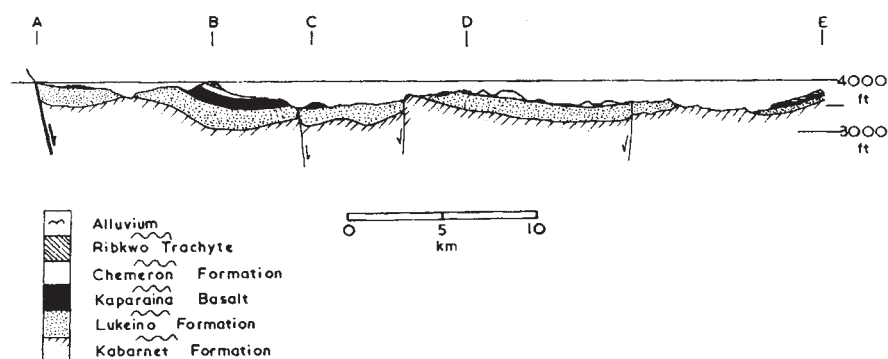


Fig. 3 North-south section through the Lukeino Formation. In the south Lukeino Formation sediment is overlain by Kaparaina Basalt, which in turn is overlain by Chemeron Formation sediment. In the north the Chemeron Formation oversteps the Lukeino Formation. A—E refer to localities indicated in Fig. 1.

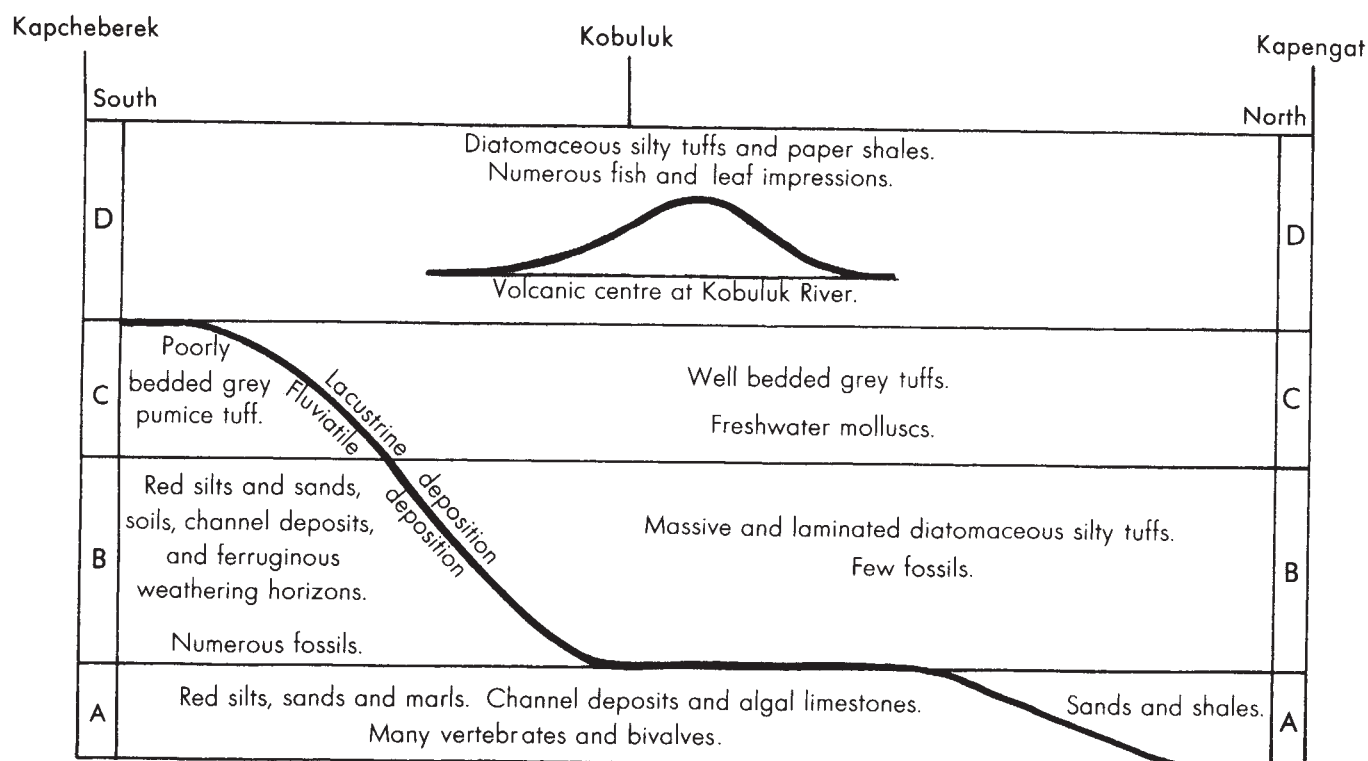


Fig. 4 Palaeoenvironments and facies distribution of the Lukeino Formation.

Basalt. The formation is exposed over an area of about 450 km<sup>2</sup> but outcrop is not continuous (Fig. 1). Sediments were derived from north, east and south, as well as from the growing Tugen Hills to the west and the Kobuluk River volcano within the basin. Much of the basal and lake margin sediment is red, and by analogy with present-day conditions, seems to be reworked from soils developed on the Kabarnet Trachyte. Algal limestones found in the red bed sequence suggest a lake marginal setting.

The grey tuffaceous sediments of Member C seem to be lacustrine in the north where they are well bedded and contain lacustrine gastropods and bivalves. In the south they are more massive, coarser and contain weathered horizons attest to emergence during Member D times, but the tuff.

Sediments of Member D were deposited in lacustrine conditions. The central parts of the basin contain abundant fish and plant remains in paper shales. Grass, leaves and diatoms (*Melosira* sp. and others) occur with cyprinids and cichlids. The fish are very abundant and scattered along bedding planes in the paper shales. Occasional suncracked horizons attest to emergence during Member D times, but these horizons are not close stratigraphically to the fish localities.

### Fauna and flora

The list of fauna collected from the Lukeino Formation (including the original Kaperyon<sup>1</sup> collections) is shown in Table 1. Fossils were recovered from 47 localities, most of which occur in Members A and B. Fossil plants include leaves, wood, grass, ferns and diatoms. Algae are common, usually occurring as algal mats. Many of the specimens await detailed study, but several additions have been made to the existing list<sup>1</sup>.

Hippopotamidae make up a large proportion of the artiodactyl assemblage while bovids, suids and giraffids are scarce. This is probably a reflection of the lacustrine and lake marginal nature of the sediments. The lake was probably always fresh or weakly saline as indicated by the

molluscs, diatoms, algae and fish. Crocodiles are common, and at least two species are present (the Nile crocodile and a new species of broadnosed crocodile (Tchernov, personal communication)).

Maglio<sup>8,9</sup> has expressed some concern over the anomalous appearance of two species of Proboscidea collected from a locality mapped as Kaperyon Formation<sup>1</sup>. Remapping has now shown that the Kaperyon Formation is composed of three separate bodies. The Proboscidea referred to above come from sediments of the Lukeino Formation and are about 1.5 Myr older than originally thought. This is in keeping with the broad 'stage of evolution' age assigned to them by Maglio.

### Age

The Lukeino Formation unconformably overlies the Kabarnet Trachyte Formation from which K-Ar dates of  $7.2 \pm 0.3$ ,  $6.8 \pm 0.2$  and  $6.7 \pm 0.3$  Myr have been obtained<sup>1</sup>. They are uncomfortably overlain by the Kaparaina Basalt Formation from which a single date of  $5.4 \pm 0.2$  Myr has been obtained<sup>1</sup>. The sediments were probably deposited about 6.5 Myr ago. Continued subsidence east of the Tugen Hills since deposition of the Ngorora Formation<sup>10</sup> (between 12 Myr and 9 Myr) resulted in several periods of sediment deposition<sup>1</sup>. The Lukeino episode covers a period about which little is known south of the Sahara, and its position in a well calibrated sequence enhances its importance.

### Hominid molar

A lower molar (Fig. 5), clearly hominoid in structure, was found a few feet from outcrops of a 0.5 m thick, pink sandy and gritty horizon with pyrolusite and ferruginous staining and concretions. The horizon lies in the middle portion of Member A at Chepboit (locality 2/219) 5.5 km west of Yatya.

At the discovery site, fossils are concentrated in the coarse basal 10 cm in which ferruginous concretions and scarlet pebbles are common. The colour of the hominid molar closely resembles that of teeth found *in situ* in the

Table 1 Faunal list, Lukeino Formation

|                   |                                                                                                                                                                           |
|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| *Mollusca         |                                                                                                                                                                           |
| *Gastropoda       | * <i>Melanoides</i> sp.<br>*2 other spp.                                                                                                                                  |
| *Bivalvia         | *2 spp.                                                                                                                                                                   |
| *Arthropoda       |                                                                                                                                                                           |
| *Ostracoda        | *undet.                                                                                                                                                                   |
| *Annelida         | *indet.                                                                                                                                                                   |
| Pisces            |                                                                                                                                                                           |
| *Clariidae        | indet.                                                                                                                                                                    |
| *Cichlidae        | * <i>Tilapia</i> sp?                                                                                                                                                      |
| *Cyprinidae       | *undet.                                                                                                                                                                   |
| *Aves             |                                                                                                                                                                           |
| Reptilia          |                                                                                                                                                                           |
| Chelonina         |                                                                                                                                                                           |
| *Pelomedusidae    | indet.                                                                                                                                                                    |
| *Trionychidae     | indet.                                                                                                                                                                    |
| *Testudinidae     | *indet.                                                                                                                                                                   |
| *Squamata         |                                                                                                                                                                           |
| *Varanidae        | *indet.                                                                                                                                                                   |
| Crocodylia        |                                                                                                                                                                           |
| *Crocodylidae     | * <i>Crocodylus niloticus</i> , Laurenti<br>* <i>Crocodylus</i> sp.                                                                                                       |
| Mammalia          |                                                                                                                                                                           |
| *Primates         |                                                                                                                                                                           |
| *Cercopithecoidea | *undet.                                                                                                                                                                   |
| *Hominoidea       |                                                                                                                                                                           |
| *Hominidae        | *undet.                                                                                                                                                                   |
| *Carnivora        |                                                                                                                                                                           |
| *Felidae          | *large sp.<br>*small sp.<br>*undet.<br>*cf. <i>Ichneumia</i> sp.<br>*cf. <i>Crocota</i> sp.<br>* <i>Enhydriodon</i> cf. <i>iluecai</i> Villalta<br>and Crusafont.         |
| *Canidae          |                                                                                                                                                                           |
| *Viverridae       |                                                                                                                                                                           |
| *Hyaenidae        |                                                                                                                                                                           |
| *Mustelidae       |                                                                                                                                                                           |
| Proboscidea       |                                                                                                                                                                           |
| Elephantidae      | <i>Stegotetrabelodon orbis</i> Maglio<br><i>Primelephas gomphotheroides</i><br>Maglio                                                                                     |
| *Deinotheriidae   | * <i>Deinotherium</i> cf. <i>bozasi</i><br>Arambourg                                                                                                                      |
| Perissodactyla    |                                                                                                                                                                           |
| Equidae           | * <i>Hipparion turkanense</i> Hooijer<br>and Maglio<br>* <i>Hipparion</i> cf. <i>sitifense</i> Pomel<br>2 spp.<br>*undet.                                                 |
| Rhinocerotidae    |                                                                                                                                                                           |
| *Chalicotheriidae |                                                                                                                                                                           |
| Artiodactyla      |                                                                                                                                                                           |
| Suidae            | <i>Nyanzachoerus tulotos</i> Cooke<br>and Ewer                                                                                                                            |
| Hippopotamidae    | <i>Hippopotamus</i> sp.<br>* <i>Giraffa</i> cf. <i>jumae</i><br>Reduncini indet.<br>*Gazellini indet.<br>Antilopini indet.<br>*?Neotragini indet.<br>*Tragelaphini indet. |
| *Tubulidentata    |                                                                                                                                                                           |
| *Orycteropodidae  | * <i>Orycteropus</i> sp. large<br>* <i>Orycteropus</i> sp. small                                                                                                          |
| Rodentia          |                                                                                                                                                                           |
| Hystricidae       | indet.                                                                                                                                                                    |
| *Other            | *indet.                                                                                                                                                                   |

\*Additions to previous list in ref. 1.

basal layer. Furthermore the tooth is slightly rolled and the roots have been dissolved away in a similar fashion to teeth found *in situ* in the grit. It is also slightly stained by pyrolusite and has a small pyrolusite concretion stuck to it. Dr P. Andrews (British Museum, Natural History) supplied the following description of the tooth.

"KNM LU335 is a left lower molar of an hominoid

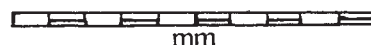


Fig. 5 KNM LU335, left lower molar, Hominidae indet.

primate. The crown was probably unerupted, and there is no sign of root development. The occlusal surface of the crown is intact but slightly pitted by weathering in places, and the base of the crown is slightly eroded so that nowhere is the basal limit present. The crown is short and broad: mesiodistal (md) length is 11.4 mm and buccolingual (bl) breadth 10.6 mm and the breadth/length index (bl/md  $\times$  100) is 93%. Talonid breadth and trigonid breadth are the same. The cusps are low and rounded, particularly the protoconid and hypoconid. The trigonid basin is small but quite deep. It is set well back from the low mesial marginal ridge of the crown and is bounded distally by a low transverse ridge connecting protoconid and metaconid. The talonid basin is small and crowded by the talonid cusps, so much so that the width of the basin is only half that of the total crown width. The length of the talonid basin is approximately equal to the lengths of the trigonid basin and distal fovea put together. The hypoconulid is placed buccal to the midline of the crown to a degree more often seen in  $M_2$  than in  $M_1$  in hominoid lower molars. The distal fovea is a deep pit distal to the low rounded hypoconulid-entoconid ridge which is cut by a very narrow groove connecting the distal fovea with the talonid basin. The groove system of the occlusal surface has a dryopithecine Y configuration. The buccal main groove and the buccal distal groove are particularly distinct, running between the buccal cusps to end in small pits on the remnants of the buccal cingulum, in the interval between cusps. Elsewhere the grooves are partly obscured by the secondary wrinkling which is most strongly developed in the talonid basin. Enamel thickness is very great.

Whether it is  $M_1$  or  $M_2$ , KNM LU335 is considerably smaller than any of the australopithecines, *sensu lato*. It is closest in general dimensions to the  $M_2$  of the chimpanzee. In morphology the Lukeino specimen is remarkably similar to the  $M_1$  of robust australopithecines<sup>8</sup>. The most characteristic feature of the crown, and contributing to its breadth, is the lateral flare of its buccal edge. Since there are also faint traces of a buccal cingulum at the bases of the buccal grooves, it seems very likely that the lateral flare is formed as a result of incorporation of the cingulum into the occlusal surface of the crown, a process that in this tooth is not quite complete. The same feature is present in the hominoid upper molar from Ngorora<sup>7</sup> which is also low crowned, has a marked lingual flare, and has faint traces of a lingual cingulum.

Another feature of the Lukeino lower molar is the



approximately equal division of the crown into central (talonid) and mesial/distal basins. The protocone of the upper molar occludes with the talonid basin, and the hypocone with the basin constituted by the distal fovea of the equivalent lower molar and the trigonid basin of the lower molar next in line. The equal division of the lower molar crown into these two occlusal basins indicates that the upper molar had approximately equal sized protocone and hypocone. This is the case with the Ngorora upper molar, but is quite different from most dryopithecines, in which the lower molar trigonid basins occupy an insignificant proportion of crown length, the talonid basins occupying up to three quarters of the crown length, and the upper molar protocones are much larger than the hypocones. This pattern seen in dryopithecines is repeated in modern apes and also in *Ramapithecus wickeri* and some australopithecines, but in general hominids have more equally sub-divided lower molars, as in the Lukeino molar. KNM LU335, is thus tentatively included within the Hominidae."

I thank the Ministry of Natural Resources of the Government of Kenya for permission to study in Baringo District;

the National Museum of Kenya for help with excavation and specimen preparation; S. Cooke, R. Villar, D. Smith, M. Fulton, A. and J. Cooper, Mr Kiptalam Chepboi and Mr Joshua Chetalam for their assistance in the field. Dr P. Andrews photographed the hominid molar and Mrs D. Andrews gave valuable assistance in Nairobi. The author presently holds a NERC research assistantship to Professor W. W. Bishop at Queen Mary College, University of London. Financial support for the fieldwork was provided by the Natural Environment Research Council and the Wenner-Gren Foundation for Anthropological Research, New York.

Received April 14; accepted June 3, 1975.

- <sup>1</sup> Bishop, W. W., Chapman, G. R., Hill, A. P., and Miller, J. A., *Nature*, **213**, 389–394 (1971).
- <sup>2</sup> Walsh, J., *Rep. Geol. Surv. Kenya*, **84**, 1–56 (1969).
- <sup>3</sup> King, B. C., and Chapman, G. R., *Phil. Trans. R. Soc.*, **A271**, 185–208 (1972).
- <sup>4</sup> Martyn, J. E., thesis, Univ. London (1969).
- <sup>5</sup> Martyn, J. E., *Nature* **215** (1967).
- <sup>6</sup> Robinson, J. T., *Transvaal Mus. mem.* **9**, 1–179 (1956).
- <sup>7</sup> Bishop, W. W., and Chapman, G. R., *Nature*, **226**, 914–918 (1970).
- <sup>8</sup> Maglio, V. J., *Nature* **225**, 328–332 (1970).
- <sup>9</sup> Maglio, V. J., *Trans. Am. phil. Soc. N.S.*, **63** (3), 1–149 (1973).
- <sup>10</sup> Bishop, W. W., and Chapman, G. R., *Nature*, **226**, 914–918 (1970).

## Noble gases in lava rock from Mount Capulin, New Mexico

E. W. Hennecke & O. K. Manuel

Nuclear Division, Department of Chemistry, University of Missouri, Rolla, Missouri 65401

*Noble gases in lava rocks from Mount Capulin crater cone contain 'parentless' <sup>40</sup>Ar and xenon trapped from the hot magma. The isotopic composition of the xenon is consistent with a mixture of 90% atmospheric and 10% solar xenon, but no radiogenic <sup>129</sup>Xe has been observed. These results do not support an earlier suggestion that radiogenic <sup>129</sup>Xe, found in CO<sub>2</sub> gas from this region of New Mexico, had been transported in hot magmas.*

THE presence of a relatively large excess of radiogenic <sup>129</sup>Xe, the decay product of <sup>129</sup>I, has been recorded<sup>1</sup> in CO<sub>2</sub> well-gas from Harding County, New Mexico. The origin of the CO<sub>2</sub> gas is not understood, and the radiogenic <sup>129</sup>Xe may have been brought near to the Earth's surface in hot magmas<sup>1,2</sup>, where intrusive contact with crustal carbonate rocks produced CO<sub>2</sub>, during a phase of recent igneous activity in this region of New Mexico<sup>3</sup>. Reports on the iodine content of limestone<sup>4</sup> and the equilibrium ratio<sup>5</sup> of <sup>129</sup>I/<sup>127</sup>I indicate however, that only a negligible fraction ( $\approx 10^{-7}$ ) of the radiogenic <sup>129</sup>Xe

in the CO<sub>2</sub> gas could have come from the limestone.

We have already suggested<sup>1</sup> that lava rocks from this region of New Mexico might contain radiogenic <sup>129</sup>Xe, provided that xenon in the magma had not completely escaped as the lava cooled or had been diluted by exchange with atmospheric gases. Radiogenic <sup>40</sup>Ar can be used as a trace<sup>6,7</sup> to estimate the amount of gas loss from the hot lava, and a comparison of the abundances of trapped noble gases with those in the atmosphere<sup>8,9</sup> can be used to look for equilibration with atmospheric noble gases. We therefore undertook this study to measure the abundance and isotopic composition of the noble gases in a lava rock from this region of New Mexico, using a large specimen ( $\approx 2$  kg) of lava rock from the Mount Capulin crater cone. The age of this cone has been estimated from <sup>14</sup>C dating to be between 4,400 and 10,000 years old<sup>10</sup>.

The abundances and isotopic compositions were measured in two samples of the lava rock. To minimise surface contamination, the samples were taken from the interior of the specimen and were not crushed before gas extraction. They were mounted in the upper chamber of a water-cooled quartz extraction

Table 1 Noble gases released from lava rock, Mount Capulin

| Sample             | I             | I           | I           | Blank I       | I                       | II            | II          | Blank II        | II                      |
|--------------------|---------------|-------------|-------------|---------------|-------------------------|---------------|-------------|-----------------|-------------------------|
| Weight (g)         | 18.164        | 18.164      | 18.164      | 1,700         | 18.164                  | 15.865        | 15.865      | 1,700           | 15.865                  |
| Temperature (°C)   | 600           | 1,200       | 1,700       |               | 800–1,700               | 800           | 1,700       |                 | 800–1,700               |
| <sup>4</sup> He    | 1.11 ± 0.01   | 1.29 ± 0.02 | 2.67 ± 0.03 | 1.32 ± 0.02   | $\approx 1.35 \pm 0.05$ | 0.84 ± 0.03   | 1.63 ± 0.06 | 1.22 ± 0.04     | $\approx 0.41 \pm 0.10$ |
| <sup>22</sup> Ne*  | 0.013 ± 0.001 | 0.58 ± 0.02 | 1.59 ± 0.05 | 0.022 ± 0.001 | 2.16 ± 0.07             | 0.33 ± 0.01   | 2.24 ± 0.02 | 0.015 ± 0.001   | 2.56 ± 0.03             |
| <sup>36</sup> Ar*  | 0.021 ± 0.001 | 0.64 ± 0.04 | 1.21 ± 0.08 | 0.020 ± 0.001 | 1.85 ± 0.12             | 0.026 ± 0.002 | 2.98 ± 0.18 | 0.0064 ± 0.0004 | 3.00 ± 0.20             |
| <sup>84</sup> Kr†  | 0.031 ± 0.001 | 0.40 ± 0.01 | 0.95 ± 0.03 | 0.017 ± 0.001 | 1.35 ± 0.04             | 0.25 ± 0.07   | 1.96 ± 0.53 | 0.0098 ± 0.0026 | 2.20 ± 0.60             |
| <sup>130</sup> Xe‡ | 0.48 ± 0.02   | 0.47 ± 0.02 | 1.63 ± 0.08 | 0.15 ± 0.01   | 2.43 ± 0.13             | 0.37 ± 0.02   | 3.34 ± 0.20 | 0.21 ± 0.01     | 3.50 ± 0.23             |
| <sup>40</sup> Ar*  |               | 3.65 ± 0.77 | 100.3 ± 7.1 |               | 104 ± 8                 | 0.54 ± 0.04   | 112 ± 7     |                 | 112 ± 7                 |

\*Units of  $10^{-9}$  cm<sup>3</sup> g<sup>-2</sup>, at STP.

†Units of  $10^{-11}$  cm<sup>3</sup> g<sup>-1</sup>, at STP.

‡Units of  $10^{-13}$  cm<sup>3</sup> g<sup>-1</sup>, at STP.

**Table 2** Isotopic compositions of noble gases released from Mount Capulin lava rocks

| Sample                               | I         | I             | I             | II            | II            | Air    | Solar  |
|--------------------------------------|-----------|---------------|---------------|---------------|---------------|--------|--------|
| Weight (g)                           | 18.164    | 18.164        | 18.164        | 15.865        | 15.865        |        |        |
| Temperature (°C)                     | 600       | 1,200         | 1,700         | 800           | 1,700         |        |        |
| <sup>20</sup> Ne/ <sup>22</sup> Ne   |           | 9.77±0.03     | 9.87±0.03     | 9.80±0.04     | 9.84±0.03     | 9.81   | 13.0   |
| <sup>21</sup> Ne/ <sup>22</sup> Ne   |           | 0.0289±0.0001 | 0.0290±0.0001 | 0.0290±0.0001 | 0.0290±0.0001 | 0.0290 | 0.033  |
| <sup>38</sup> Ar/ <sup>36</sup> Ar   |           | 0.184±0.002   | 0.188±0.002   | 0.188±0.001   | 0.187±0.001   | 0.187  | 0.185  |
| <sup>40</sup> Ar/ <sup>36</sup> Ar   |           | 301.2±1.1     | 378.4±2.0     | 316.3±1.0     | 333.0±0.9     | 295.5  | ≤0.6   |
| <sup>78</sup> Kr/ <sup>84</sup> Kr   |           |               | 0.0062±0.0001 |               | 0.0065±0.0002 | 0.0062 | 0.0064 |
| <sup>80</sup> Kr/ <sup>84</sup> Kr   |           | 0.0394±0.0005 | 0.0400±0.0003 | 0.0398±0.0002 | 0.0396±0.0005 | 0.0396 | 0.0401 |
| <sup>82</sup> Kr/ <sup>84</sup> Kr   |           | 0.200±0.001   | 0.202±0.001   | 0.201±0.001   | 0.201±0.001   | 0.202  | 0.202  |
| <sup>83</sup> Kr/ <sup>84</sup> Kr   |           | 0.200±0.001   | 0.202±0.001   | 0.201±0.001   | 0.201±0.001   | 0.202  | 0.202  |
| <sup>86</sup> Kr/ <sup>84</sup> Kr   |           | 0.306±0.001   | 0.304±0.001   | 0.305±0.001   | 0.305±0.001   | 0.305  | 0.304  |
| <sup>124</sup> Xe/ <sup>130</sup> Xe |           |               | 0.0239±0.0004 |               | 0.0243±0.0003 | 0.0235 | 0.0273 |
| <sup>126</sup> Xe/ <sup>130</sup> Xe |           |               | 0.0224±0.0003 |               | 0.0226±0.0002 | 0.0221 | 0.0261 |
| <sup>128</sup> Xe/ <sup>130</sup> Xe |           |               | 0.478±0.005   |               | 0.483±0.004   | 0.470  | 0.506  |
| <sup>129</sup> Xe/ <sup>130</sup> Xe | 6.45±0.03 | 6.45±0.03     | 6.49±0.03     | 6.51±0.04     | 6.47±0.02     | 6.48   | 6.32   |
| <sup>131</sup> Xe/ <sup>130</sup> Xe | 5.17±0.06 | 5.16±0.03     | 5.17±0.02     | 5.20±0.05     | 5.16±0.03     | 5.19   | 5.01   |
| <sup>132</sup> Xe/ <sup>130</sup> Xe | 6.57±0.05 | 6.60±0.03     | 6.55±0.01     | 6.59±0.05     | 6.53±0.03     | 6.59   | 6.13   |
| <sup>134</sup> Xe/ <sup>130</sup> Xe | 2.56±0.01 | 2.55±0.02     | 2.52±0.01     | 2.45±0.04     | 2.54±0.02     | 2.56   | 2.29   |
| <sup>136</sup> Xe/ <sup>130</sup> Xe | 2.18±0.01 | 2.17±0.01     | 2.14±0.01     | 2.16±0.01     | 2.16±0.02     | 2.17   | 1.87   |

The isotopic compositions of atmospheric Ne, Ar, Kr and Xe are from refs 33, 12, 34 and 35, respectively, and the isotopic compositions of solar Ne, Ar, Kr and Xe are from ref. 29.

bottle, the pressure was reduced to about  $1 \times 10^{-8}$  mmHg and the system blank was measured by collecting and analysing the gases generated by heating the empty molybdenum crucible to the highest extraction temperature, about 1,700 °C. After the system blank had been reduced to an acceptable level, the sample was dropped into the degassed crucible and the gases were extracted by stepwise heating. Extraction temperatures of 600 °C, 1,200 °C and 1,700 °C were used for sample I (18.164 g) and 800 °C and 1,700 °C were used for sample II (15.864 g). The samples were maintained at each temperature for 30 min. The gases released at each temperature were cleaned, separated and analysed using procedures described previously<sup>11</sup>. Standard volumes of air ( $\approx 0.01$  cm<sup>3</sup> at standard temperature and pressure—STP) were analysed before and after the analysis of each sample to determine the sensitivity and mass discrimination of the mass spectrometer.

### Noble gas abundances

The amounts of noble gases released at each extraction temperature are shown in Table 1, together with the uncertainties in the gas abundance measurements resulting from variations in the sensitivity of the mass spectrometer. The mass spectrometer signals for the 1,700 °C system blanks have been converted to effective gas contents (cm<sup>3</sup> g<sup>-1</sup>, at STP). The isotopic ratios of the blanks were atmospheric to within  $\pm 5\%$ , and blanks at 600, 800, and 1,200 °C were negligible except with <sup>4</sup>He when, at 600, 800 and 1,200 °C they were about 0.75 of their value at 1,700 °C. The relatively high blank level for He probably results from the diffusion of He into the glass vacuum system during the extraction and cleaning of gases. The total content of each noble gas, corrected for blanks, is shown in Table 2. In computing the totals we considered only the <sup>4</sup>He released at 1,700 °C, since the amounts released at lower temperatures were approximately equal to the <sup>4</sup>He blank level. The results of these analyses provide no information on the fraction of <sup>4</sup>He which is radiogenic, so we shall denote the concentration thus: (<sup>4</sup>He).

The amounts of radiogenic <sup>40</sup>Ar (<sup>40</sup>rAr) were computed by assuming non-radiogenic argon to be of atmospheric composition<sup>12</sup> (Table 1). The average concentration of <sup>40</sup>rAr is  $108 \times 10^{-9}$  cm<sup>3</sup> per gram of lava rock at STP. Decay of <sup>40</sup>K during the lifetime of the rock would generate only  $28 \times 10^{-9}$  cm<sup>3</sup> per g of potassium (STP). We interpret this as evidence of incomplete loss of <sup>40</sup>rAr from the magma. These results suggest that radiogenic <sup>129</sup>Xe from the magma would also be trapped in the lava rocks, and its detection depends on the extent of dilution by atmospheric xenon.

To look for evidence of exchange of atmospheric noble

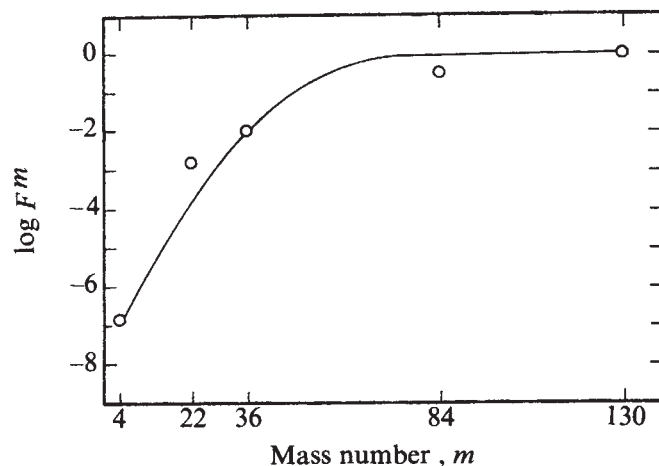
gases with those in the lava rocks, we compared the abundance pattern of trapped noble gases in the lava rock with that in the atmosphere, using the method of Canals *et al.*<sup>13</sup>, who defined a fractionation factor,  $F^m$ , as

$$F^m = ({}^m\text{X}/{}^{130}\text{Xe})_{\text{sample}} / ({}^m\text{X}/{}^{130}\text{Xe})_{\text{cosmic}} \quad (1)$$

where <sup>m</sup>X is any noble gas isotope of mass number,  $m$ , and cosmic abundances are those given by Suess and Urey<sup>14</sup>.

The values of  $\log F^m$  against mass number are shown in Fig. 1 for noble gases in the lava rocks (Table 1) and in the atmosphere<sup>15</sup>. Relative to the atmospheric abundances of noble gases, the average value of  $F^m$  in the lava rocks for <sup>84</sup>Kr is lower by a factor of 3, and for <sup>22</sup>Ne it is higher by a factor of 15. Within experimental uncertainties, the  $F^m$  values of <sup>38</sup>Ar and (<sup>4</sup>He) are identical to those in the atmosphere. The irregular variation of  $F^m$  values with  $m$  (Fig. 1) does not fit the pattern that could be expected if equilibration with atmospheric gases is attained. Solubility equilibrium of atmospheric gases with the hot magma would have discriminated against the incorporation of large atoms<sup>16</sup>, and adsorption equilibrium of atmospheric gases would have discriminated against small atoms<sup>17</sup>. The high Xe/Kr ratios in lava rock cannot be accounted for by the solution of atmospheric xenon in the parent magma, but may have resulted from adsorption.

**Fig. 1** Log fractionation factor against mass number. The solid line shows the abundance pattern of noble gases in the atmosphere<sup>15</sup>; ○ noble gases in the Mount Capulin samples.



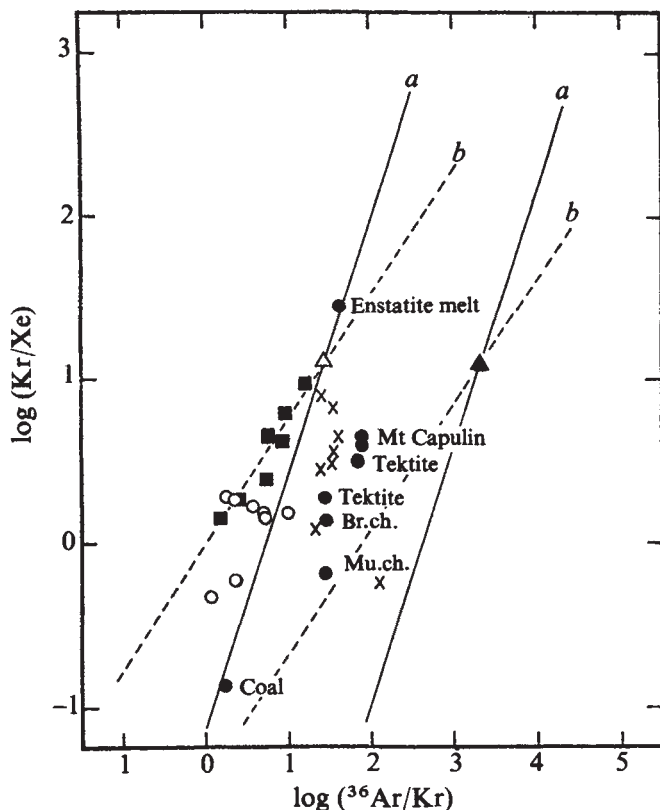


Fig. 2 A comparison of noble gas ratios in the Mount Capulin samples and in other material. *a*, Ratios expected in rocks which sample atmospheric and cosmic abundances of noble gases; *b*, slope used by Fisher<sup>9,21</sup> and Phinney<sup>18</sup>; ●, coal<sup>13</sup>, Murray carbonaceous chondrite (Mu. ch.)<sup>20</sup>, Bruderheim chondrite (Br. ch.)<sup>32</sup>, Thailand tektites<sup>8</sup>, Mount Capulin lava rocks, and enstatite melt<sup>16</sup>; ○, shales<sup>13</sup>; ■, basalts<sup>9</sup> from Pacific Seamount and Nigeria; ×, basalts from mid-ocean ridge reported to contain primordial noble gases<sup>20-22</sup>; △, air<sup>15</sup>; ▲, cosmic<sup>14</sup>.

Stepwise extraction of gases is useful in separating gases from different sites<sup>8,11</sup>, as adsorbed gases are preferentially released at the lower extraction temperatures, and the dissolved gases are enriched in gases released at higher extraction temperatures. Adsorption is highly specific for xenon<sup>18</sup>, and the enrichment of xenon in the 600 °C fraction of sample I may result from the release of adsorbed gases. The bulks of all noble gases were, however, retained together with radiogenic <sup>40</sup>Ar until the samples were melted at 1,700 °C. As <sup>40</sup>Ar was derived from the magma, the release of this isotope can be considered as a tracer for the release of noble gases incorporated into the rock from the magma. Although we cannot rule out completely the possibility of tenaciously adsorbed xenon being released in the 1,700 °C fraction, high Xe/Kr ratios can be expected in the magma as the results of other studies<sup>13,17,18</sup> indicate that the Xe/Kr ratio in air is anomalously low because of the preferential adsorption of xenon on fine-grained sediments. Since 'parentless' <sup>40</sup>Ar came in with the hot magma and is still present in the lava rock, there is an overwhelming probability that any radiogenic <sup>129</sup>Xe originally in the hot magma would also still be trapped in the lava rock.

The relatively high values of the Ne/Ar ratios in these lava rocks seem to confirm reports of a high Ne/Ar ratio in pumice<sup>19</sup> and of high Ne/Ar ratios in lava rocks from the sea floor<sup>20</sup>. Although the enrichment of lighter noble gases in the latter rocks has been interpreted as evidence of the presence of primordial rare gases from the Earth's mantle<sup>20-22</sup>, it is possible that the enrichment results from the preferential leakage of lightweight atmospheric noble gases into rocks which have low concentrations of indigenous noble gases. Thailand tektites show a large excess of neon<sup>8</sup>, but it has been shown,

from the diffusion coefficient of neon in tektite glass<sup>23</sup> and from the Kr-Ar<sup>23,24</sup> and fission track<sup>25</sup> age estimates of those tektites, that the high neon content could result from the diffusion of atmospheric neon into the tektite. The isotopic ratios of Ne and Xe in the atmosphere are quite different from those in meteorites<sup>26</sup> and from that imbedded in lunar fines<sup>27</sup>.

### Isotopic abundances

The isotopic composition of the noble gases released from Mount Capulin basalt are given in Table 2. No attempt has been made to measure the <sup>3</sup>He content, and there have been no corrections for blanks, because most of the isotopic ratios are essentially atmospheric. The isotopic compositions are atmospheric within  $\pm 2\sigma$  ( $\sigma = 1$  standard deviation), except for an obvious excess of radiogenic <sup>40</sup>Ar and perhaps a slight depletion of the heavy xenon isotopes in the 1,700 °C fraction of sample I and a slight enrichment of the light xenon isotopes in the 1,700 °C fraction of sample II.

We could not find radiogenic <sup>129</sup>Xe in lava rock from the region where radiogenic <sup>129</sup>Xe was found in CO<sub>2</sub> gas; however, the 'parentless' <sup>40</sup>Ar and high Xe/Kr ratios in gases released at the highest extraction temperatures compel us to conclude that the Xe originated in the magma. The absence of radiogenic <sup>129</sup>Xe in these samples suggests that radiogenic <sup>129</sup>Xe in the CO<sub>2</sub> gas may come from some source other than the hot magma, and since it did not come from the decomposition of limestone<sup>1</sup>, other origins should be considered. Leakage of CO<sub>2</sub> from an asthenosphere which contains a CO<sub>2</sub>-rich liquid phase<sup>28</sup> seems to be a reasonable alternative which could account for both the high CO<sub>2</sub> content of the gas and the presence of the decay product of extinct <sup>129</sup>I in gas pockets trapped within the Earth's crust.

The isotopic ratios (Table 2) contain no evidence of solar-type noble gases, except for the slight enrichment of the light Xe isotopes and depletion of the heavy Xe isotopes in the 1,700 °C fractions. A mixture containing atmospheric Xe and solar Xe in the ratio 9:1, could account for the isotopic ratios in Xe released from both samples at 1,700 °C. Because of the very small differences between atmospheric Xe and that released from the Mount Capulin basalt at 1,700 °C, caution should be exercised in interpreting our results as evidence of primitive Xe within the Earth. The isotopic ratios of the lighter noble gases seem to be atmospheric.

### Atmospheric or solar origin?

Previous observations cited as evidence that oceanic lava rocks contain primitive gases from the Earth's mantle include the similarity between the relative abundances of noble gases in some lava rocks and in meteorites<sup>20-22</sup>, the strong contrast of these relative abundances with those in the atmosphere<sup>20,21</sup>, the fact that the simple fractionation of atmospheric noble gases could not simultaneously produce higher values for the Ne/Xe ratio and lower values for the Kr/Xe ratio, and the failure of noble gas ratios in some lava rocks to lie along equilibrium correlation lines defined by Henry constants<sup>16,30</sup> for the solubility of atmospheric noble gases into a melt<sup>20-22</sup>.

Fisher<sup>9</sup> first used the correlation of noble gas ratios for <sup>36</sup>Ar, Kr, and Xe on log-log plots to differentiate between noble gases from the atmosphere and noble gases from a solar (primordial) source. Subsequently, Dymond and Hogan<sup>20</sup> and Fisher<sup>21</sup> used these plots, and one involving Ne, to show that the noble gas contents in some deep sea lava rocks did not fit the equilibrium correlation line for atmospheric noble gases. For any four noble gases, a, b, c, and d, the concentration ratios,  $C_a/C_b$ ,  $C_c/C_d$ , and so on, in a melt at equilibrium with an atmosphere having partial pressures,  $p_a$ ,  $p_b$ ,  $p_c$  are defined by<sup>9,16,30</sup>

$$\ln(C_a/C_b) - \ln(p_a/p_b) = [(r_a^2 - r_b^2)/(r_c^2 - r_d^2)] [\ln(C_c/C_d) - \ln(p_c/p_d)] \quad (2)$$

where  $r_a$  is the atomic radius of the noble gas, a, and so on.



Figures 2 and 3 compare the noble gases in the Mount Capulin lava rocks with the equilibrium lines defined by equation (2) for noble gases from the Earth's atmosphere<sup>15</sup> and from an atmosphere containing cosmic abundances of noble gases<sup>14</sup>. Values of atomic radii given by Pauling<sup>31</sup> were used. The figures give the noble gas ratios used by Fisher<sup>9,21,22</sup> and by Dymond and Hogan<sup>20</sup> to distinguish primordial from atmospheric gases. Since the slope which we calculated from equation (2) for the plot of  $\log(\text{Kr/Xe})$  against  $\log(^{36}\text{Ar/Kr})$  is significantly steeper than that shown by Fisher<sup>9,21</sup> and Phinney<sup>18</sup>, and since differences in the slopes may lead to different conclusions about the origin of noble gases in certain samples, their value for the slope has been shown (Fig. 2). Experimental values<sup>16</sup> for the solubility of He, Ne, and Ar in enstatite melts, and extrapolated values<sup>16</sup> for the solubility of Kr and Xe, have been used to calculate the noble gas ratios in a melt which sampled gases from the atmosphere, and these ratios agree with the solid equilibration lines (Fig. 2a).

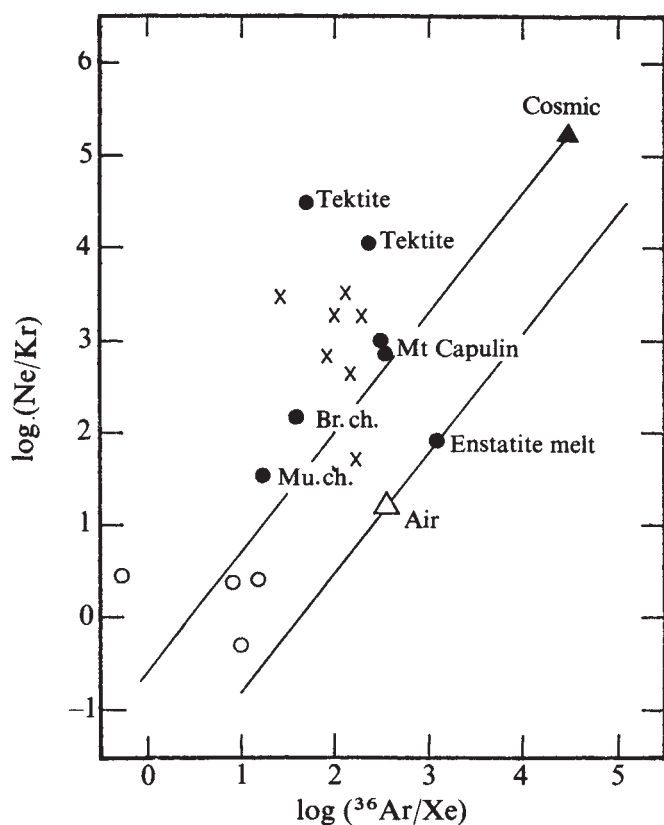


Fig. 3 A comparison of noble gas ratios in the Mount Capulin lava rocks and in other material. The solid lines were calculated from equation (2) and define the noble gas ratios in samples which have equilibrated with gases having atmospheric<sup>15</sup> and cosmic<sup>14</sup> abundances. Symbols as in Fig. 2.

All of the noble gas ratios in Fig. 2 represent terrestrial samples, except for two data points representing the Bruderheim chondrite<sup>32</sup> and the Murray carbonaceous chondrite<sup>26</sup>. The ratios of these three heavier noble gases in terrestrial samples lie on both sides of the atmospheric equilibration line, with the gases in three shale samples<sup>13</sup> and the coal sample<sup>13</sup> lying close to that expected from equilibration with atmospheric gases. Noble gases in seven basalt samples analysed by Fisher<sup>9</sup> are generally to the left of the atmospheric equilibration line, and noble gases in tektites<sup>8</sup>, in samples from the Vema Fracture Zone of the Atlantic<sup>21,22</sup>, in glassy outer portions of basalts

from mid-ocean ridge<sup>20</sup>, and in the Mount Capulin basalts are generally to the right of the atmospheric equilibration line. One view of the data shown in Fig. 2 is that those terrestrial samples lying between the atmospheric and the cosmic equilibration lines may have sampled a mixture of atmospheric and primordial gases within the Earth<sup>20-22</sup>.

All of the non-radiogenic noble gases, including Ne, have been included in Fig. 3. Fewer data are presented there because of the absence of Ne analyses on the coal, several shale samples, or basalts from the Vema Fracture Zone of the Atlantic. The noble gas ratios in the Mount Capulin basalts, as well as in the Bruderheim and the Murray chondrite, lie close to the ratios to be expected if equilibrium with cosmic noble gases had been obtained. All of the noble gas ratios in the terrestrial samples shown in Fig. 3, however, lie to the left of the air equilibration line, and many are shifted far to the left of the line which defines equilibration with cosmic abundances of noble gases. In view of the fact that the terrestrial samples, which lie to the left of the cosmic equilibration line in Fig. 3, lie between the atmospheric and cosmic equilibration lines in Fig. 2, it seems that the position of these noble gas ratios in Fig. 2 does not necessarily indicate equilibration of the melts with a mixture of primordial and atmospheric gases<sup>20-22</sup>. Rather, it seems that the low Kr/Xe ratios shown in Fig. 2 may result from the fact that the Kr/Xe ratio for the Earth is appreciably lower than the Kr/Xe ratio for the atmosphere, as has been noted earlier<sup>13</sup>, and the high Ne/Kr ratios shown in Fig. 3 may result from the preferential leakage of Ne into igneous rocks which form near the Earth's surface and have very low amounts of indigenous noble gases.

These analyses reveal the presence of a large excess of 'parent-less' <sup>40</sup>Ar, though radiogenic <sup>129</sup>Xe was not observed. The abundance pattern of gases released at the highest extraction temperatures suggests that there has been little or no exchange of atmospheric xenon with these gases. The absence of radiogenic <sup>129</sup>Xe in basalts which have trapped <sup>40</sup>Ar from the magma implies that this is not the source of radiogenic <sup>129</sup>Xe in CO<sub>2</sub> gas wells.

This investigation was supported financially by the National Science Foundation.

Received February 17; accepted June 2, 1975.

- 1 Boulos, M. S., and Manuel, O. K., *Science*, **174**, 1334 (1971).
- 2 Bulter, W. A., Jeffrey, P. M., Reynolds, J. H., and Wasserburg, G. J., *J. geophys. Res.*, **68**, 3283 (1963).
- 3 Levorsen, A. I., *Geology of Petroleum* (edit. by Berry, F. A. F.), 220 (Freeman, San Francisco, 1967).
- 4 Becker, V. J., Bennett, J. H., and Manuel, O. K., *Chem. Geol.*, **9**, 133 (1972).
- 5 Srinivasan, B., Alexander, E. C., Jr, and Manuel, O. K., *Science*, **173**, 327 (1971).
- 6 Dalmayrle, G. B., and Moore, J. G., *Science*, **161**, 1132 (1968).
- 7 Funkhouser, J. G., Fisher, D. E., and Bonatti, E., *Earth planet. Sci. Lett.*, **5**, 95 (1968).
- 8 Hennecke, E. W., Manuel, O. K., and Sabu, D. D., *J. geophys. Res.* (in the press).
- 9 Fisher, D. E., *Earth planet. Sci. Lett.*, **9**, 331 (1970).
- 10 Muehlberger, W. R., *Bull. geol. Soc. Am.*, **66**, 1600 (1955).
- 11 Hennecke, E. W., and Manuel, O. K., *Z. Naturf.*, **26a**, 1980 (1971).
- 12 Nier, A. O., *Phys. Rev.*, **77**, 789 (1950).
- 13 Canals, R. A., Alexander, E. C., Jr, and Manuel, O. K., *J. geophys. Res.*, **73**, 3331 (1968).
- 14 Suess, H. E., and Urey, H. C., *Rev. mod. Phys.*, **28**, 48 (1956).
- 15 Verniani, F., *J. geophys. Res.*, **71**, 385 (1966).
- 16 Kirsten, T., *J. geophys. Res.*, **73**, 2807 (1968).
- 17 Fanale, F. P., and Cannon, W. A., *Earth planet. Sci. Lett.*, **11**, 362 (1971).
- 18 Phinney, D., *Earth planet. Sci. Lett.*, **16**, 413 (1972).
- 19 Lord Rayleigh, *Proc. R. Soc.*, **A170**, 451 (1939).
- 20 Dymond, J., and Hogan, L., *Earth planet. Sci. Lett.*, **20**, 131 (1973).
- 21 Fisher, D. E., *Nature*, **244**, 344 (1973).
- 22 Fisher, D. E., *Geophys. Res. Lett.*, **1**, 161 (1974).
- 23 Reynolds, J. H., *Geochim. cosmochim. Acta*, **20**, 101 (1960).
- 24 Zähringer, J., and Gentner, W., *Nature*, **199**, 583 (1963).
- 25 Gentner, W., Storz, D., and Wagner, G. A., *Geochim. cosmochim. Acta*, **33**, 1075 (1969).
- 26 Reynolds, J. H., *Phys. Rev. Lett.*, **4**, 351 (1960).
- 27 The Lunar Samples Preliminary Examination Team, *Science*, **165**, 1211 (1969).
- 28 Green, H. W., III, *Nature*, **238**, 1 (1972).
- 29 Srinivasan, B., Hennecke, E. W., Sinclair, D. E., and Manuel, O. K., *Proc. third Lunar Science Conference, Geochim. cosmochim. Acta*, suppl. 3(2), 1927 (1972).
- 30 Blander, M., Grimes, W. R., Smith, N. V., and Watson, G. M., *J. phys. Chem. Wash.*, **63**, 1164 (1959).
- 31 Pauling, L., *The Nature of the Chemical Bond*, 514 (Cornell University Press, Ithaca, 1960).
- 32 Manuel, O. K., and Rowe, M. W., *Geochim. cosmochim. Acta*, **28**, 1999 (1964).
- 33 Eberhardt, P., Eugster, O., and Marti, K., *Z. Naturf.*, **20a**, 623 (1965).
- 34 Nief, G., *Isotopic Abundance Ratios Given for Reference Samples Stocked by the National Bureau of Standards* (edit. by Mohler, F.), 51 (National Bureau of Standards, Washington, DC, 1960).
- 35 Nier, A. O., *Phys. Rev.*, **79**, 450 (1950).

# Existence and possible roles of transcriptional barriers in T7 DNA early region as shown by electron microscopy

Jean-Luc Darlix & Mireille Horaist

Service de Biochimie (142), Département de Biologie CEN Saclay, BP 2 91190 Gif/Yvette, France

*Transcription of T7 DNA in vitro by Escherichia coli RNA polymerase in the absence or presence of termination factor  $\rho$  has been studied extensively using electron microscopy. It is demonstrated that sequences located between the early genes behave as transient transcriptional barriers in the absence of  $\rho$  and are recognised both as  $\rho$ -dependent terminators and as RNaseIII cleavage sites.*

In the current model for selective transcription, initiation and termination of RNA transcripts are dictated by specific signals on the DNA template that are recognised and translated into biochemical events by the transcription apparatus.

Specific initiation of RNA synthesis has been studied extensively *in vitro* using a model 'transcription of T7 DNA by *E. coli* RNA polymerase' that requires no added factor other than DNA and RNA polymerase holoenzyme<sup>1</sup>. It has been shown that a subunit of RNA polymerase holoenzyme, the  $\sigma$  factor, governs the tight binding of RNA polymerase molecules<sup>1</sup> to three specific sites within the early promoter region of T7 DNA (ref. 2), from which region RNA chain initiation takes place very rapidly in the presence of ribonucleoside triphosphates<sup>3-5</sup>. Darlix *et al.*<sup>6</sup> found that the subsequent elongation of RNA is discontinuous, that is, during the transcription of a long DNA sequence, regions of rapid transcription alternate with regions of slow transcription. More precisely, previous investigations have clearly indicated that the discontinuities in the transcription process are caused by sequences present only in natural DNAs and probably located between genes<sup>7</sup>, and that such sequences in the T7 DNA early region could well include  $\rho$ -dependent termination sites<sup>7</sup>.

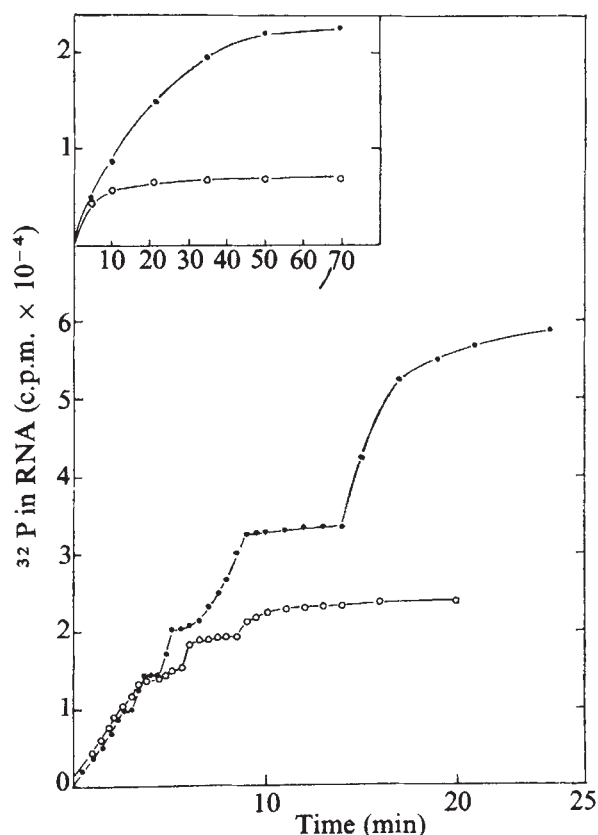
We describe here the discontinuous propagation of *E. coli* RNA polymerase on T7 DNA template, as well as the action of  $\rho$  on this process, as observed by electron microscopy.

## Discontinuous transcription of T7 DNA

Discontinuous transcription of DNA is best observed when the RNA chain growth rate is reduced; this is obtained by decreasing the concentration of one nucleotide<sup>8</sup>. As previously shown<sup>7</sup>, transcription of T7 DNA with or without  $\rho$  is clearly discontinuous in conditions in which GTP concentration is lowered to  $1.5 \times 10^{-5}$  M. Successive plateaux occur during the kinetics of total RNA synthesis in the presence or absence of  $\rho$ . We have located the growing RNA on the physical map of T7 DNA at times corresponding to the plateaux (Fig. 1).

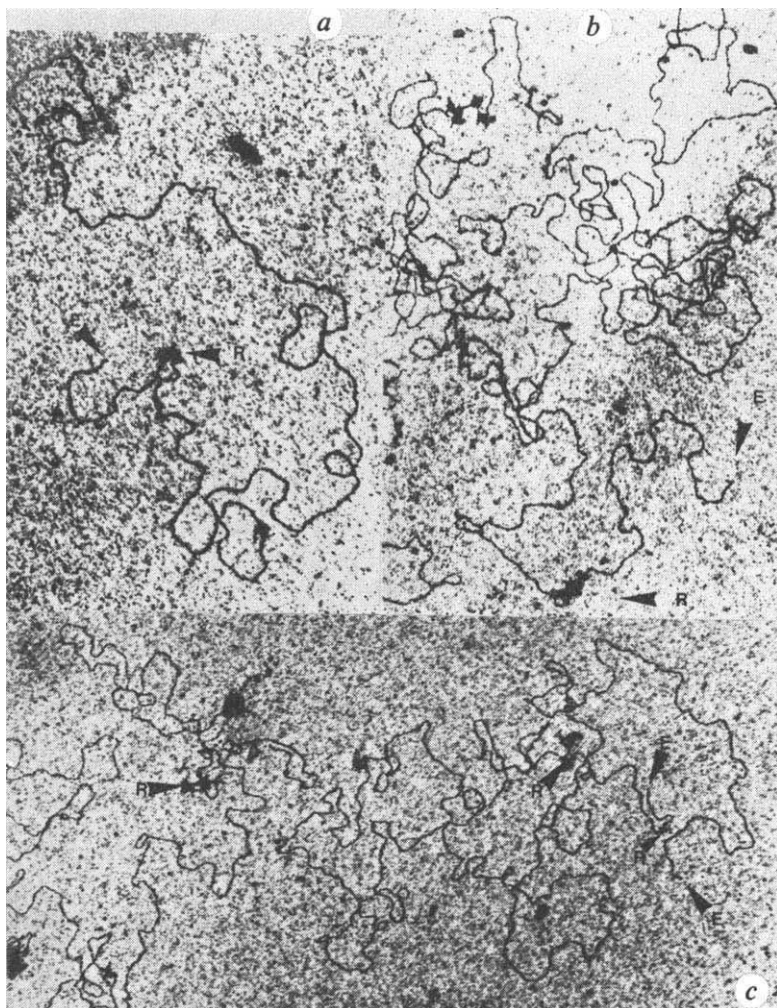
The DNA-RNA polymerase-RNA transcription complexes have been studied in the electron microscope by dark-field observation of specimens prepared by adhesion on positively charged carbon films<sup>8</sup>. In the present conditions and in the absence of  $\rho$ , growing RNA chains ap-

pear as clustered bushes on the DNA (Fig. 2). If transcription is stopped after 4, 11 and 25 min, that is, when RNA synthesis is particularly low (plateaux, Fig. 1), most active enzymes are found at or near the end of gene 0.3—the first one<sup>9</sup>—(Fig. 3a), the end of genes 0.3 and 0.7 (Fig. 3b), and the end of genes 0.7, 1 and 1.1 (Fig. 3c), respectively. Previous results of the RNA chain length analysis of the corresponding transcription products agree with the present findings<sup>7</sup>. In fact, the mRNA of gene 0.3, of genes 0.3 and 0.7, and of genes 0.3, 0.7 and eventually 1 and 1.1



**Fig. 1** Discontinuous transcription of T7 DNA *in vitro*. T7 DNA, *E. coli* RNA polymerase and factor  $\rho$  were prepared as described previously<sup>7</sup>. The reaction mixture contained 52  $\mu$ g T7 <sup>3</sup>H-DNA (4,000 c.p.m.  $\mu$ g<sup>-1</sup>), 10  $\mu$ g RNA polymerase (50–70% active based on the specific activity reported by Burgess<sup>11</sup>), 7  $\mu$ g  $\rho$  when added (○), 0.1 mM ATP, CTP,  $\alpha$ -<sup>32</sup>P-UTP (300 mCi mmol<sup>-1</sup>), 0.015 mM GTP and salt conditions as described previously<sup>7</sup>, final volume 2.5 ml. Where indicated, 75- $\mu$ l aliquots were withdrawn and RNA recovered by acid precipitation. Insert, general aspect of the kinetics of RNA synthesis with T7 DNA template at limiting GTP concentration. In these conditions, the relative molar ratios of RNA polymerase to  $\rho$  and T7 DNA are 6:14:1 assuming respective molecular weights of 490,000, 250,000 and  $25 \times 10^6$ . ●, RNA made in absence of  $\rho$ ; ○, in presence of  $\rho$ .





**Fig. 2** Discontinuous transcription observed using electron microscopy. Conditions of transcription are those in Fig. 1. The samples were diluted to a concentration of  $0.1$  to  $0.5 \mu\text{g ml}^{-1}$  T7 DNA with an ice-cold buffer (that of transcription) and specimens prepared as described previously<sup>8</sup>. Observations were made with a Siemens Elmiskop 101 electron microscope. The dark field was obtained by the tilted beam method and a thin-film objective aperture of  $20 \mu\text{m}$  was used. Micrographs were recorded on  $65 \times 90 \text{ mm}$  Kodak films at magnification ranging from  $13,000$ – $40,000\times$ . *a*, After 4 min transcription, small RNA bushes are visible. Magnification:  $\times 80,000$ . E, DNA end; R, RNA bushes. *b*, After 11 min transcription, two DNA molecules are spread in the field and several RNA bushes are clustered at the end of gene 0.7. Magnification:  $\times 65,550$ . *c*, After 4 min transcription in conditions which all four nucleotides were present at  $0.1 \text{ mM}$ . One entire DNA molecule is spread in the field and most RNA bushes visible are at the end genes. Small RNA bushes may be seen near one DNA end. Magnification:  $\times 51,300$ .

were synthesised after, respectively, 4, 10 and 20 min of transcription at low GTP concentration. Consequently, sequences located between genes in the T7 DNA early region behave as transient transcriptional barriers. In addition, transcribing RNA polymerases also stop in the middle of gene 1, indicating the presence of a transcriptional barrier in this region<sup>7</sup>. In the present conditions total RNA synthesis ceases after 50 min and the major product is the polycistronic mRNA of T7 DNA early region as usual<sup>7</sup> (Fig. 1, insert).

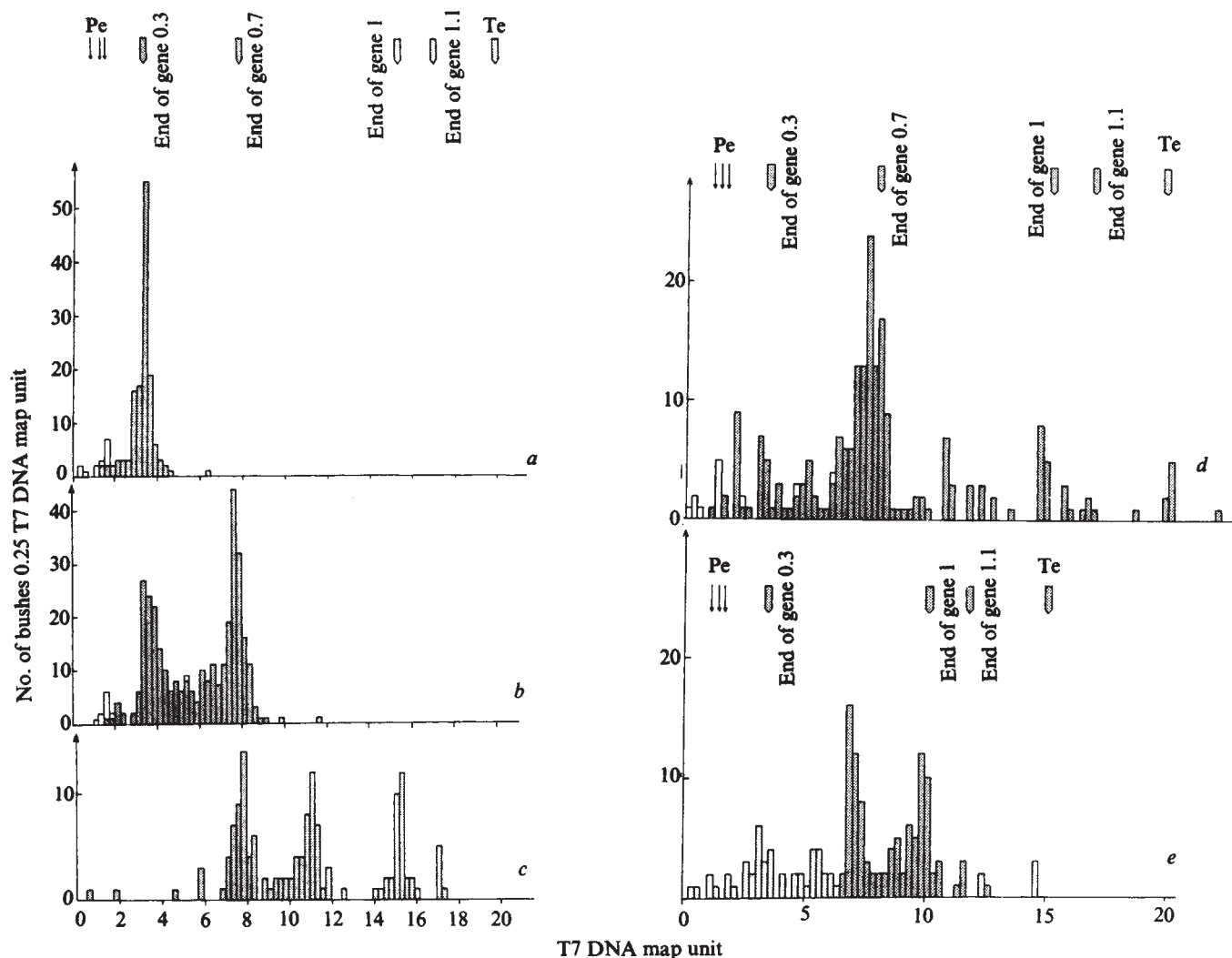
On the basis of the above data we can estimate the relative times devoted to high and low rates of transcription and RNA chain growth rate. During 25 min of transcription, efficient RNA synthesis occurred during approximately 8 min (Fig. 1); thus a third of the time is devoted to efficient synthesis. These 8 min should be considered as a maximum, for the first 2 min of RNA synthesis are devoted to initiation (result not shown). As DNA sequences of 2,500 to 6,500 nucleotides have been transcribed within these 6–8 min of efficient synthesis (Fig. 3c), RNA chain growth rate varies from 5 to 18 nucleotides  $\text{s}^{-1}$ . Furthermore, in 3 min (third to fourth plateau, Fig. 1) RNA polymerase molecules can transcribe a sequence 3,600 nucleotides long, that of genes 1 and 1.1 (Fig. 3b and c), then at a chain growth rate of 20 nucleotides  $\text{s}^{-1}$ . Transcription of gene 0.7 proceeds at the same speed (second to third plateau, only a small fraction of enzymes have reached the end of gene 0.7 at 7 min; result not shown). Finally, assuming that all enzymes transcribe the genes at 20 nucleotides  $\text{s}^{-1}$ , the most efficient have read four genes (0.3 to 1.1; 5.5 min required) after 25 min synthesis, whereas

the less efficient enzymes have read only two genes (0.3 and 0.7; 2 min required).

On the other hand, transcription of the sequences located at the end and/or beginning of the early genes seems to proceed slowly and last a long time. If we estimate that the length of each transcriptional barrier does not exceed 190–285 nucleotides ( $0.50$ – $0.75$  T7 DNA map unit, Fig. 3 and ref. 10), the corresponding RNA chain growth rate should be about  $10$ – $60$  nucleotides  $\text{min}^{-1}$ .

Thus all these calculations show that, at limiting GTP concentration, efficient transcription may well correspond to the rapid and brief synthesis of specific RNAs and low rate of transcription to the slow growth of short and particular RNA sequences<sup>4,7,10</sup>. A similar transcription pattern can be drawn from results of experiments in which the limiting nucleotide was CTP or UTP instead of GTP (results not shown). If the present conclusions are independent of the low concentration used for one nucleotide, a large fraction of the polymerases should also be found between genes after a given period of synthesis carried out at  $50$ – $100$  nucleotides  $\text{s}^{-1}$ , that is, when the concentration of each nucleotide is  $0.1 \text{ mM}$  (ref. 6). In fact, after 4 min transcription with T7 DNA template, growing RNA transcripts are clustered near the end of genes, mostly at the end of gene 0.7 (Fig. 3d). Chain length analysis of the transcription product agree with the present findings, as most RNA are 2,300–3,000 nucleotides long corresponding to the transcription of genes 0.3 and 0.7 (results not shown). At a chain growth rate of  $50$ – $100$  nucleotides  $\text{s}^{-1}$  (ref. 6), transcription of genes 0.3 and 0.7 should have required 30–60 s of the 4-min synthesis; thus the relative time de-





**Fig. 3** Histograms of the positions of the transcription complexes. Experiments were carried out as described in Figs 1 and 2. Interesting features of the micrographs taken were redrawn on paper at  $\times 10$  magnification using an Automega enlarger. Length measurements were made on these drawings with a map ruler. One T7 DNA map unit corresponds to 380 base pairs. Mapping was according to Simon and Studier<sup>9</sup>. *a*, After 4 min transcription, 146 RNA bushes were on 62 DNA molecules. 58 free DNA ends observed. *b*, After 11 min transcription, 328 RNA bushes were on 135 DNA molecules. 100 free DNA ends. *c*, After 25 min transcription, 140 RNA bushes were on 53 DNA molecules. 50 free DNA ends. *d*, After 4 min transcription in conditions in which all four nucleotides were at 0.1 mM, 230 RNA bushes were on 79 DNA molecules. 70 free DNA ends. *e*, As in *d* but the template used was T7 C18 DNA, 153 RNA bushes were on 50 DNA molecules. 40 free DNA ends. Open areas, complexes in which RNA is hardly visible; stippled areas, complexes in which RNA is clearly visible.

voted to rapid transcription is roughly the same whether GTP concentration is limiting or not, and equal to 10–25% of the total transcription time.

With T7 C18 DNA template (in which regions of gene 0.7 and between 0.7 and 1 are deleted<sup>9</sup>) most transcribing RNA polymerases are not located in the same regions of the T7 DNA physical map, but again are still close to the end of genes and in the middle of gene 1 (Fig. 3e). After 15 min transcription and in conditions in which the RNA polymerase is in large excess, the growing RNA transcripts are mostly at the end of genes<sup>13</sup>.

### Transcriptional barriers associated with termination of RNA synthesis

We have shown above that regions located between genes behave as transient transcriptional barriers. In the absence of  $\rho$ , spontaneous termination of RNA synthesis seldom occurs at these sites<sup>13</sup> and they are transcribed. The double-stranded RNA that results is further digested by

RNaseIII during the processing of the mRNA of the T7 DNA early region<sup>4,10,14,15</sup>.

Addition of factor  $\rho$  to the transcription apparatus does not modify the binding of RNA polymerase to the DNA (ref. 2). As shown by electron microscopy, at respective molar ratios of RNA polymerase to  $\rho$  and T7 DNA of 6:14:1, three to four enzymes bind to the three to four sites located in the early promoter region at 1.1, 1.41, 1.65 and 0.5 units of T7 DNA physical map<sup>2</sup> (Figs 4 and 5). RNA synthesis very probably starts from these sites<sup>7</sup> and proceeds discontinuously in the presence of  $\rho$  (Fig. 1). In conditions of limiting GTP concentration, inhibition of transcription caused by  $\rho$  begins after 4–5 min; at that time most active enzymes have transcribed gene 0.3 and are between genes 0.3 and 0.7, where factor  $\rho$  specifically terminates transcription for the first time<sup>7</sup>. After 11 min of synthesis, termination of transcription intervenes once more between genes 0.3 and 0.7 and between genes 0.7 and 1. Transcription with  $\rho$  is completed after 20 min (Fig. 1, insert) and the major products are monocistronic mRNA of gene 0.3 (plus a part of the early promoter region) and of

gene 0.7 in a molar ratio 4 : 1 (Figs 4 and 5 of ref. 7).

The action of  $\rho$  may be seen clearly in Figs 4b and 6a, which show the localisation of RNAs still attached to the DNA after 11 min of transcription. The number of RNA bushes per T7 DNA molecule is two- to threefold less with termination factor  $\rho$  than without; RNA bushes are no longer clustered (Fig. 4b). An unusual number of RNA polymerases that have just initiated transcription are present in the regions of the early promotor and end of genes 0.3 and 0.7 (Fig. 6a). Remaining RNAs are not at the end of genes 0.3 and 0.7, but located between early promotor and the end of gene 0.7 and primarily within gene 0.3. Transcription complexes are also present between genes 0.3 and 0.7 indicating that  $\rho$  is not completely efficient and/or part of the sequences at the end of genes are transcribed in the presence of  $\rho$ . Similar conclusions can be drawn from the distribution pattern of the RNA bushes after 4 min transcription with  $\rho$  in the presence of saturating concentration of the four nucleotides (Fig. 6c, for control see Fig. 3d).

Our findings establish certain aspects of the proposed mechanism for T7 DNA transcription with  $\rho$ . Transcription starts within the early promotor region, very probably at the three binding sites; data presented in Fig. 6 support the idea that reinitiation of transcription at the early promotor and at the beginning of genes 0.7 and 1 (ref. 16) follows the termination of transcription maximised by  $\rho$  at the end of genes 0.3 and 0.7 (refs 7, 13, 16). As there is no preferential degradation of one or more early T7 mRNA *in vivo*, our data clearly indicate a possible way in which transcription of the T7 early genes are modulated *in vivo*<sup>17-19</sup>. RNA transcripts made in the presence of  $\rho$

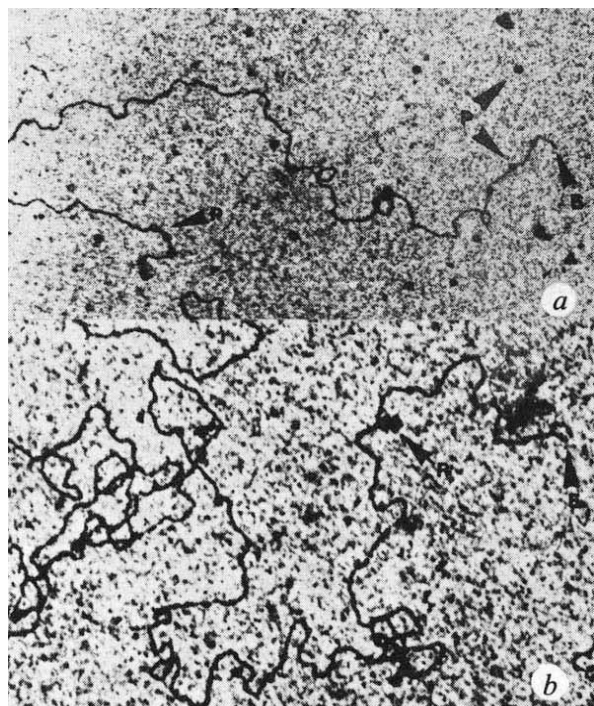


Fig. 4 Transcription of T7 DNA in presence of factor  $\rho$  observed using electron microscopy. *a*, Binding complexes. Conditions of binding were as reported in Fig. 1 except that nucleotides were absent. After 5 min incubation at 37°C dilution of the samples and specimen preparation were carried out as described in Fig. 1 but at 37°C instead of 0-4°C. Two individual DNA ends are visible with RNA polymerase molecules either bound near DNA end or in the background of the film. Magnification:  $\times 79,160$ . *b*, Transcription complex. Conditions of transcription with  $\rho$  were as reported in Fig. 1. A typical RNA bush bound to DNA is shown. Magnification:  $\times 93,800$ . E, DNA end; R, RNA bush; P, RNA polymerase.

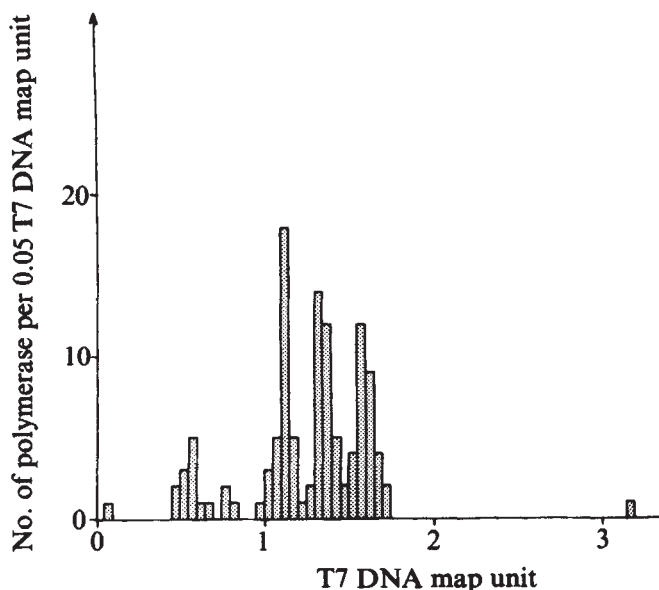


Fig. 5 Position of the RNA polymerase binding sites in T7 DNA early promotor region in presence of  $\rho$ . Specimens were prepared and analysed as described in Figs 3 and 4. Histogram of the positions of 114 RNA polymerase molecules on 38 T7 DNA molecules. One T7 DNA unit corresponds to 380 base pairs.

are processed by RNaseIII to give monocistronic mRNA of T7 DNA early region identical to those found *in vivo* and in similar relative ratios<sup>7</sup>.

Finally, the action of  $\rho$  between genes is well shown in Fig. 6b. After transcription without  $\rho$  and at limiting GTP concentration,  $\rho$  is added (plateau, Fig. 1). One minute later the synthesis is stopped and the distribution pattern of the growing RNA chains determined. Almost all RNA transcripts clustered at the end of genes 0.3 and 0.7 have disappeared (70% total RNA chains), whereas those within gene 0.3 remain (for control, see Fig. 3b).

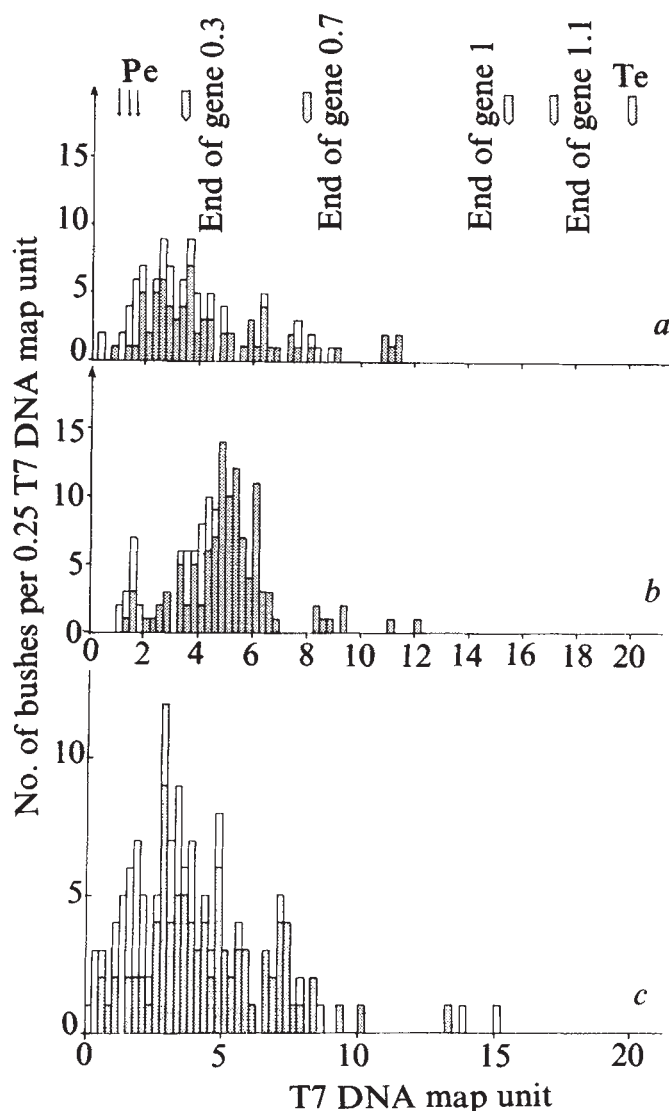
## Conclusions

It now seems possible to draw the following picture for the *in vitro* synthesis of a polycistronic mRNA such as that of the T7 DNA early region. Once RNA chain initiation is completed, intense bursts of transcription of long DNA sequences, the genes, alternate with the slow synthesis of short and particular RNA sequences which takes a long time to complete.

On the basis of our experiments and on those of others (for review see ref. 1), we may well understand the mechanism of *in vitro*  $\rho$ -dependent termination of transcription.  $\rho$  Termination is directly related to the decrease in RNA chain growth rate at the end of genes and in the middle of gene 1. Furthermore, efficiency of  $\rho$  terminations is also related to the time necessary for RNA polymerase to read through a transcriptional barrier. When the mean chain growth rate is decreased by limiting concentration of one nucleotide, transcription beyond gene 0.7 does not occur, or at least is extremely low<sup>7</sup>. In addition, all attempts made to demonstrate the presence of  $\rho$ -specific binding sites on T7 DNA, as on fd DNA<sup>20</sup>, were unsuccessful. Intervention of  $\rho$  during transcription may well seem to be a probable and transient interaction between this factor and slowly transcribing RNA polymerases, the conformation of which has been modified by a transcriptional barrier.

Such T7 DNA sequences, which include  $\rho$ -active sites, also coincide with the corresponding RNaseIII cleavage sites, of the RNA transcript<sup>4,7,14</sup>. Data now available show that of these DNA sequences some should be (dA)<sub>n</sub>: (dT)<sub>n</sub>-rich<sup>10,21</sup> and other (dC)<sub>n</sub>: (dG)<sub>n</sub>-rich<sup>10</sup>, and expected to be





**Fig. 6** Positions of the transcription complexes in presence of  $\rho$ . Experiments were carried out as in Figs 1–3. *a*,  $\rho$  added together with RNA polymerase, and transcription was stopped after 11 min. 120 RNA bushes were on 96 DNA molecules, 100 free DNA ends observed. *b*,  $\rho$  was added after 10 min transcription, itself stopped 1 min later. 121 RNA bushes were on 64 DNA molecules. 110 free DNA ends. *c*,  $\rho$  was added together with RNA polymerase, and transcription was carried out in conditions in which all four nucleotides were at 0.1 mM. 140 RNA bushes were on 100 DNA molecules. 100 free DNA ends. Open areas, complexes in which RNA is hardly visible; stippled areas, complexes in which RNA is clearly visible.

preceded by a specific sequence –G–C–C–U–U–A–U– and followed by –G–A–U– (ref. 22).

More generally, similar transcriptional barriers probably exist in all natural DNA as indicated by the heterogeneous distribution of the RNA made in the course of transcription with T4,  $\phi$ 80,  $\lambda$ , *E. coli* and calf thymus DNA templates<sup>7</sup>, and these barriers exert a control on transcription in the presence or absence of an additional factor<sup>1</sup>. We have shown termination of T7 DNA transcription under the control of  $\rho$ ; this process has also been described with various natural DNAs but not with artificial templates<sup>1</sup> and thus seems to be a general phenomenon requiring specific DNA sites and  $\rho$ . Another termination event occurs at the end of the early region of T7 DNA and is  $\rho$  independent<sup>23</sup>; the barrier located at 20% of the T7 DNA physical map, however, does not stop all transcriptions and its efficiency decreases with increasing concentration of salt<sup>16</sup>. Such transcriptional barriers are not rare and exist within the late region of T7 DNA<sup>23</sup>, and also within  $\lambda$  and *E. coli* genomes<sup>24,25</sup>.

Finally, transcriptional barriers can be located very close to the promoter region of a bacterial operon, and could thus be important in the control of transcription of one or more structural genes. In the course of transcription of the tryptophan operon, RNA polymerase is frequently rejected at a specific site ahead of the first structural gene, but a protein factor was found to antagonise the abortive synthesis of tryptophan mRNA<sup>26</sup>.

We thank C. Bardin for assistance; I. Pinset, J. Dubochet and M. Schweiger for suggestions; B. Haas, A. Sentenac for reading the manuscript; and P. Fromageot for his interest.

Received February 27; accepted May 29, 1975.

- <sup>1</sup> Chamberlin, M. J., *A. Rev. Biochem.*, **43**, 721–775 (1974).
- <sup>2</sup> Bordier, C., and Dubochet, J., *Eur. J. Biochem.*, **44**, 617–624 (1974).
- <sup>3</sup> Chamberlin, M. J., and Ring, J., *J. molec. Biol.*, **70**, 221–237 (1972).
- <sup>4</sup> Dunn, J. J., and Studier, F. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1559–1563 (1973).
- <sup>5</sup> Darlix, J. L., and Dausse, J. P., *FEBS Lett.*, **50**, 214–218 (1975).
- <sup>6</sup> Darlix, J. L., Sentenac, A., and Fromageot, P., *Biochim. biophys. Acta*, **166**, 438–449 (1968).
- <sup>7</sup> Darlix, J. L., *Biochimie*, **56**, 693–703 (1974).
- <sup>8</sup> Dubochet, J., Ducommun, M., Zollinger, M., and Kellenberger, P., *J. Ultrastruct. Res.*, **35**, 147–167 (1971).
- <sup>9</sup> Simon, M. N., and Studier, F. W., *J. molec. Biol.*, **79**, 249–265 (1973).
- <sup>10</sup> Darlix, J. L., and Fromageot, P., *Biochimie*, **56**, 703–710 (1974).
- <sup>11</sup> Burgess, R. R., *J. biol. Chem.*, **244**, 6160–6169 (1969).
- <sup>12</sup> Delius, H., Westphal, H., and Axelrod, N., *J. molec. Biol.*, **74**, 677–687 (1973).
- <sup>13</sup> Minkley, E., *J. molec. Biol.*, **83**, 305 (1974).
- <sup>14</sup> Dunn, J. J., and Studier, F. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3296–3300 (1973).
- <sup>15</sup> Darlix, J. L., *Eur. J. Biochem.*, **51**, 369–376 (1975).
- <sup>16</sup> Darlix, J. L., *Eur. J. Biochem.*, **35**, 517–526 (1973).
- <sup>17</sup> Summers, W. C., Brunovskis, I., and Hyman, R. W., *J. molec. Biol.*, **74**, 291–300 (1973).
- <sup>18</sup> Ponta, H., et al., *Molec. gen. Genet.*, **134**, 281–297 (1974).
- <sup>19</sup> Minkley, E., *J. molec. Biol.*, **83**, 289–304 (1974).
- <sup>20</sup> Oda, T., and Takanami, M., *J. molec. Biol.*, **71**, 799–802 (1972).
- <sup>21</sup> Gomez, B., and Lang, D., *J. molec. Biol.*, **70**, 239–251 (1972).
- <sup>22</sup> Rosenberg, M., Kramer, A. R., and Steitz, J. A., *J. molec. Biol.*, **89**, 777–782 (1974).
- <sup>23</sup> Golomb, M., and Chamberlin, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 760–764 (1974).
- <sup>24</sup> Lebowitz, P., Weissman, S. M., and Radding, C. M., *J. biol. Chem.*, **246**, 5120–5139 (1971).
- <sup>25</sup> Ikemura, T., and Dahlberg, J. E., *J. biol. Chem.*, **248**, 5033–5041 (1973).
- <sup>26</sup> Pannkoek, H., Brammar, W. J., and Pouwels, P. M., *Molec. gen. Genet.* (in the press).

## letters to nature

### Hard X-ray observations near Ariel 1118 – 61

OBSERVATIONS made by Ariel V in the region of Cen X-3 have revealed a new transient X-ray source with a periodicity of 6.75 min (refs 1 and 2). Tentative identifications are: with a Mira-type variable star by Fabian *et al.*<sup>3</sup>, who suggest the 6.75 min periodicity is associated with the rotation period of a compact companion; and with a heavily reddened, variable

ultraviolet star<sup>4</sup>. As well as the proportional counter observations during the period 1974 December 17 to 1975 January 31, the Imperial College crystal scintillator, sensitive to the photon energy range 26 keV to 1.2 MeV, viewed the same region of the sky. This detector has a sensitive area of 8 cm<sup>2</sup>, an opening angle of 8° FWHM defined by an active collimator and is offset from the spacecraft spin axis by an angle of 3°. A source a few degrees from the direction of the spin axis can be detected



by determining the modulation in the counting rate seen in four equal angle spin sectors with a time resolution of 512 s. A single channel (50–190 keV) mode with a 48 s time resolution was also used. Since a spurious modulation is induced in the apparatus by cosmic rays, two pointing direction offsets on opposing sides of the source have to be used, and the X-ray contribution is obtained by a subtraction technique.

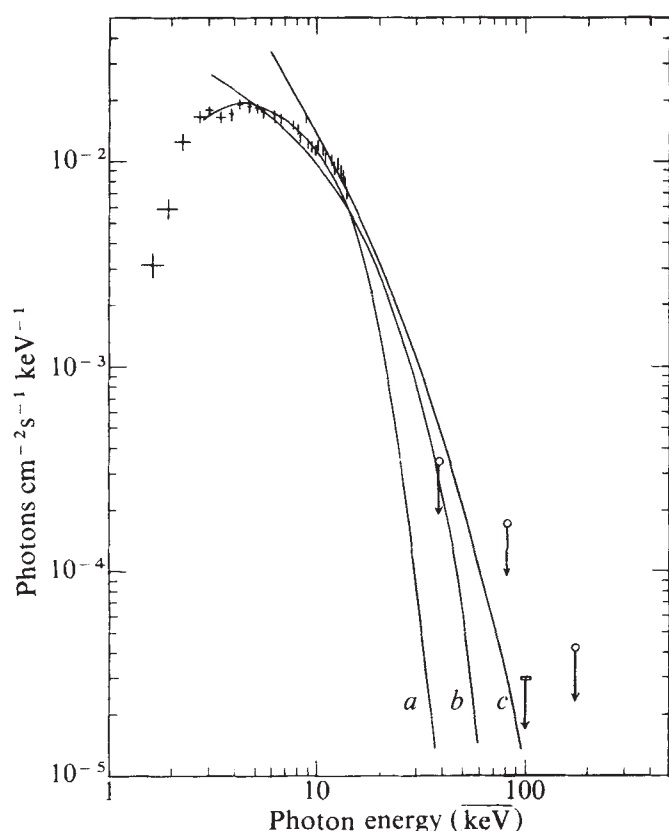
Scheduled observations of Cen X-3 coincided with an extended low from 1974 December 17 to 1975 January 10 and no detectable signal was seen above statistical noise. The offsets were, however, equally good for putting upper limits on the hard flux of A1118–61 (Table 1).

**Table 1** Upper limits on A1118–61 flux

| Period              | 2 $\sigma$ upper limits (photons cm <sup>-2</sup> s <sup>-1</sup> keV <sup>-1</sup> ) |                      |                      |
|---------------------|---------------------------------------------------------------------------------------|----------------------|----------------------|
|                     | 40 keV                                                                                | 80 keV               | 180 keV              |
| 1974 December 19–22 | $3.4 \times 10^{-4}$                                                                  | $1.8 \times 10^{-4}$ | $4.0 \times 10^{-5}$ |
| 1975 January 8–11   | $5.4 \times 10^{-4}$                                                                  | $1.7 \times 10^{-4}$ | $7.8 \times 10^{-5}$ |

To compare these results with those reported at lower energies, we note that Ives *et al.*<sup>2</sup> find the spectral shape varies little during their observations using proportional counters. We can therefore scale the proportional counter spectrum given by these workers, according to the light curves of references 1 and 2 to obtain the low energy spectrum corresponding to our periods of observations. Figure 1 shows the result of this procedure with the proportional counter points reduced by a factor of 2.3 to correspond to the epoch 1974 December 19–22. Our 2 $\sigma$  scintillator upper limits are plotted at the logarithmic

**Fig. 1** Four independent, hard X-ray upper limits plotted at 2 $\sigma$  for the spectrum of A1118–61 together with low energy X-ray data normalised to the same epoch (1974 December 19–22). Three theoretical fits to the data are also shown: *a*, 3 keV black body spectrum; *b*, 7 keV black body spectrum modified by electron scattering; *c*, 20 keV thermal bremsstrahlung spectrum. +, proportional counter data; ○, offset mode; □, search for 6.75 min period.



mean energy of the channels and are virtually independent of the assumed spectral slope within the channel.

A further independent upper limit for the  $6.750 \pm 0.006$  min periodicity (J. Bell Burnell, personal communication) has been obtained using observations with the 48 s time resolution mode made in the period 1975 January 19–27. These data, which are less sensitive to the cosmic ray induced background, were folded with the quoted period. A 2 $\sigma$  upper limit for the depth of modulation of  $3 \times 10^{-5}$  photons cm<sup>-2</sup> s<sup>-1</sup> keV<sup>-1</sup> was obtained. This value, which has been normalised to a photon energy of 100 keV, is almost independent of the assumed spectral shape within the energy window for a range of likely cosmic spectra. Assuming there is essentially 100% modulation as indicated in reference 2, the above limit must be approximately halved for comparison with the time averaged data. But since the intensity may be a factor of two lower for this observation than for the standard epoch (that is, 1974 December 19–22) we plot  $3 \times 10^{-5}$  photons cm<sup>-2</sup> s<sup>-1</sup> keV<sup>-1</sup> as the corrected 2 $\sigma$  upper limit in Fig. 1.

In discussing the physical processes for the emission implied by the combined spectral measurements, it is clear that any power law spectrum fitted above 10 keV must be steeper than  $E^{-3}$ , although the published low energy data enabled even an  $E^{-1}$  fit. Thus there is no immediate reason to consider a synchrotron source. Instead, consider accretion on to a compact stellar object in a binary system. Indeed we note that the rapid spectral cutoff below 4 keV suggests strong local gas absorption<sup>3</sup>. The dominant mechanism for emission of X rays from the gas clouds accreting on to the compact object could be either (a) a black body radiation where photon scattering by electrons is unimportant, or (b) a modified black body radiation at a higher temperature with a significant skin depth so that photons must random walk. We may further modify (b) by adding a high temperature bremsstrahlung tail (free-free emission) due to inner regions of the accretion cloud shining through at harder X-ray energies. Our best fit to hypothesis (a) using a photon spectrum

$$(dI/dE)_{BB} \propto E^2 / (\exp(E/kT) - 1)$$

yields  $kT = 3 \pm 1$  keV and this is plotted in Fig. 1 as curve *a*. Fitting a modified black body spectrum (curve *b*) to the proportional counter data using

$$(dI/dE)_M \propto (dI/dE)_{BB} [gE^{-3}(1 - \exp(-E/kT))]^{1/2}$$

with the Gaunt factor  $g \propto E^{-0.4}$  gives  $kT = 7$  keV  $\pm 1$  keV. But plotting this spectrum in Fig. 1 results in a curve which is a poor fit to the lowest in energy of the scintillator counter 2 $\sigma$  upper limits and does not satisfy the proportional counter data so well. A thermal bremsstrahlung spectrum (*c*) with  $(dI/dE)_{TB} \propto E^{-1.4} \exp(-E/kT)$  and  $kT \sim 20$  keV is a plausible fit to the higher energy proportional counter data but is similarly inconsistent with the hard X-ray data. We conclude that the addition of scintillator X-ray data to the A1118–61 observations suggests that if a dominant, single source mechanism is sought, black body radiation from a low skin depth gas is the most likely.

G. F. CARPENTER  
M. J. COE  
A. R. ENGEL  
J. J. QUENBY

Physics Department,  
Imperial College,  
London SW7 2AZ, UK

Received May 14; accepted June 17, 1975.

- Eyles, C. J., Skinner, G. K., and Willmore, A. P., *Nature*, **254**, 577 (1975).
- Ives, J. C., Sanford, P. W., and Bell-Burnell, S. J., *Nature*, **254**, 578 (1975).
- Fabian, A. C., Pringle, J. E., and Webbink, R. F., *Nature*, **255**, 208 (1975).
- Chevalier, C., and Ilovaisky, S. A., *IAU Circ. No. 2778*, May 9 (1975).
- Felten, J. E., and Rees, M. J. *Astr. Astrophys.*, **17**, 226 (1972).

## Radio emission from Cyg X-3

THE most recent radio outburst of Cyg X-3 reported<sup>1,2</sup> occurred in May, 1974. Following the announcement by Ryle<sup>1</sup> of high activity we observed part of this outburst at 4.8 GHz with the 100-m telescope of the MPIfR at Effelsberg from May 22 to May 31, during a general survey of the Cygnus X region. The observed flux densities are summarised in Fig. 1 and have typical errors of 3% or less; the general behaviour of the sources is also sketched in

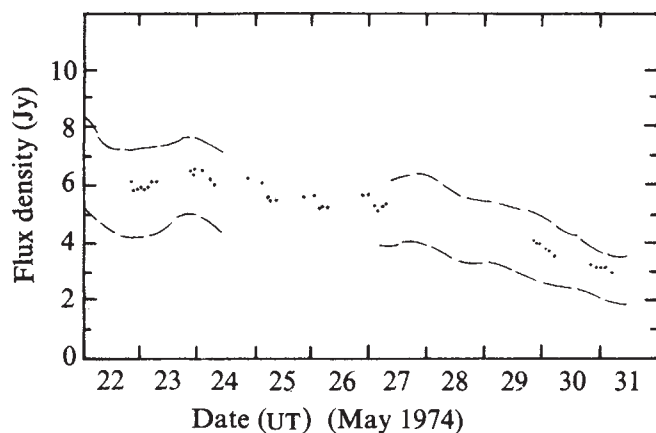


Fig. 1 Light curve of Cyg X-3 at 4.8 GHz (filled circles). The dashed lines sketch the data from Seaquist *et al.*<sup>2</sup>

for the data given by Seaquist *et al.*<sup>2</sup>. The observations were obtained as a flux density ratio with respect to NGC7027 from direct adjacent crosscuts, assuming a value of 5.8 Jy for NGC7027 (from refs 3 and 4). As our data did not contain very extended polarisation measurements and as they are consistent with the interpolated polarisation properties given in ref. 2, all points were brought to the mean flux density level using a polarisation angle of 70° and a linear polarisation of 4.7%.

Three important results emerge from our study. First, the source also shows the linear decay of long time averaging over the missing time interval of ref. 2. (For a discussion of related problems see ref. 5.) Second, the periodicity indicated in the detailed flux density behaviour is also visible in our data. Moreover we have the impression that this periodicity becomes less pronounced when progressing from May 22 to May 31. Third, we emphasise that it may be dangerous to infer a 'linear' spectrum from only two points. In Fig. 2 several spectra are shown constructed from the data of

| Table 1 Older data for Cyg X-3 |                 |                  |           |
|--------------------------------|-----------------|------------------|-----------|
| Date                           | Frequency (GHz) | Upper limit (Jy) | Telescope |
| 1964                           |                 |                  |           |
| July 4                         | 2,695           | 2.0              | NRAO 26 m |
| July 5                         | 2,695           | 2.0              |           |
| July 26                        | 2,695           | 2.0              |           |
| July 31                        | 2,695           | 2.0              |           |
| 1965                           |                 |                  |           |
| January 13                     | 4,930           | 4.0              | NRAO 26 m |
| January 14                     | 4,930           | 4.0              |           |
| January 20                     | 4,930           | 4.0              |           |
| 1967                           |                 |                  |           |
| May 31                         | 2,695           | 0.3              | NRAO 45 m |

ref. 2 and our observations. There is a strong indication that the assumption of optically thin properties may not be valid even during the decay of this outburst. This does not seem to be a systematic calibration effect between the two sets of observations.

Forcing our data down to an interpolated value on the 'linear' spectra would require us to assume for NGC7027 at 4.8 GHz a flux density value of 5.3 Jy or less which seems completely out of the question according to the spectrum given by Higgs<sup>3</sup> and Ross and Seaquist<sup>4</sup>. From a few flux density ratio measurements NGC7027/3C48 we derived a value of  $5.4 \pm 0.3$  Jy for 3C48 which is compatible with an interpolated value used in ref. 2. Thus, during the time May 22 to May 31 the spectra may be interpreted as showing the effects of optical depth (see ref. 6). A possible explanation of this behaviour may be that the sum of the recent outbursts has accumulated enough thermal plasma on the outskirts of the source for absorption now to occur even at frequencies up to several GHz.

The activity of Cyg X-3 has been quite high during the past few years and it is almost possible to say that the source underwent an outburst once every month. The source was visible for about a couple of weeks at an appreciable flux density level during each flare. So the question of past activities of the source may be important for all explanations of the nature of the source. One of us (H.J.W.) has obtained many observations of the area during regular surveys of the

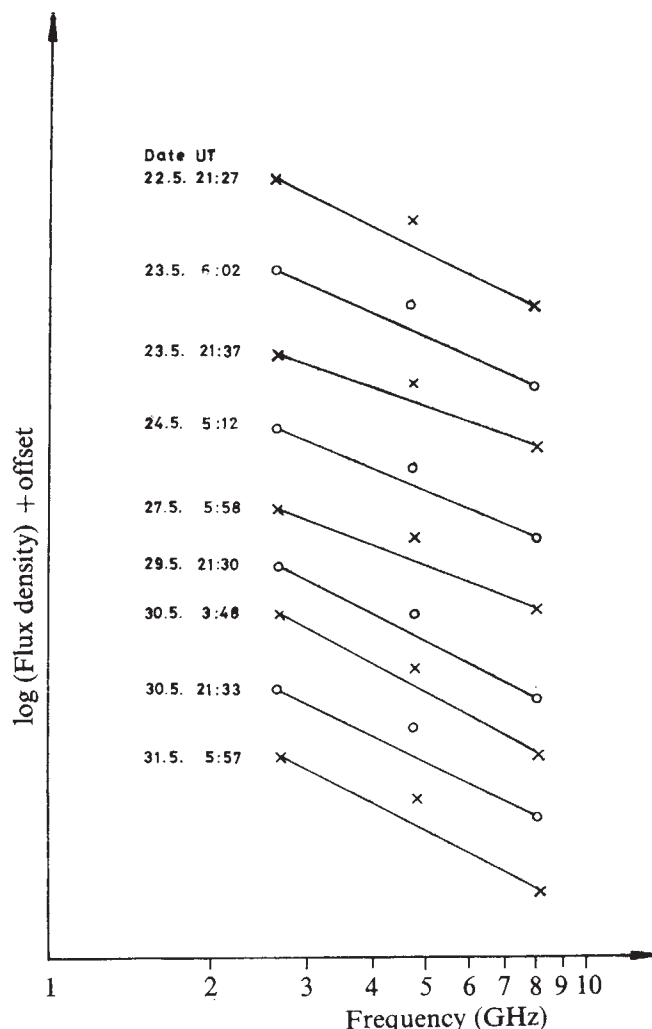


Fig. 2 Spectra of Cyg X-3 on the dates indicated.

Cygnus X region<sup>7,8</sup>; activity at the present level had a high chance of being discovered. But the data would have been regarded as showing a spurious source. So we re-examined the old records. The dates of observations and the derived upper limits ( $3\sigma$ ) are given in Table 1. Especially during July 1964 and January 1965, outbursts such as, for example, those occurring in September 1972 and in May 1974 would easily have been seen. We are tempted to conclude that ten years ago the source was not so active as today.

H.-D. BOHNENSTENGEL  
K. HENNING  
H. J. WENDKER

Hamburger Sternwarte,  
Gojenbergsweg 112,  
2050 Hamburg 80, West Germany

Received May 27; accepted June 4, 1975.

<sup>1</sup> Ryle, M., *IAU Circ. No. 2670* (1974).

<sup>2</sup> Seaquist, E. R., et al., *Nature*, **251**, 394-396 (1974).

<sup>3</sup> Higgs, L. A., *Publ. Astrophys. Branch*, **1**, 1-454 (1971).

<sup>4</sup> Ross, H. N., and Seaquist, E. R., *Mon. Not. R. astr. Soc.*, **170**, 115-119 (1975).

<sup>5</sup> Marsh, K. A., Purton, C. R., and Feldman, P. A., *Astrophys. J.*, **192**, 697-701 (1974).

<sup>6</sup> Gregory, P. C., and Seaquist, E. R., *Astrophys. J.*, **194**, 715-723 (1974).

<sup>7</sup> Wendker, H. J., *Veröff. astr. Inst. Univ. Münster*, No. 10 (1966).

<sup>8</sup> Wendker, H. J., *Astr. Astrophys.*, **4**, 378-386 (1970).

## The 'spectra' of the solar cycle and of data for Atlantic tropical cyclones

TROPICAL cyclones (including hurricanes) are significant climatic features which affect the South and East USA as well as areas of the western Atlantic, the Caribbean, and the Gulf of Mexico. Since they are considered to be a mechanism which limits the build-up of heat and energy in tropical regions<sup>1</sup>, cyclones are necessarily related to large scale circulation patterns which may be global in extent. Thus, if a relationship is to be found between solar activity and large scale meteorological phenomena, we may expect that it will evidence itself in analyses of data on cyclone occurrence and the length of the cyclone season. Here we report results of such a study, and provide evidence which is consistent with the hypothesis that a relationship exists between the solar cycle and the occurrence of Atlantic tropical cyclones.

The data sets analysed<sup>2,3</sup> relate to tropical cyclones which originated in the Atlantic region during each month from 1871 to 1973 (see ref. 2 for a discussion of the sources and quality of the cyclone data). Both the number of cyclones which occurred in each year, and the length of the annual cyclone season in months (counted as the number of months from the month of the first cyclone to the month of the last cyclone, inclusive), were extracted from the data and were smoothed with a 7-yr running mean filter (Fig. 1a, b). Also shown in Fig. 1 are the 12-month running mean sunspot numbers<sup>4,5</sup> (Fig. 1c). It was necessary to smooth the cyclone data to ensure that scatter in the data did not cause the maximum entropy spectral analysis (MESA) technique (based on an autoregressive description of the data by Burg (unpublished); see also ref. 6) to fail to give acceptable spectral estimates<sup>7</sup>.

MESA spectra for the smoothed number of Atlantic tropical cyclones and the smoothed length of the cyclone season are shown in Fig. 2a and b. For comparison, the MESA spectrum for the 12-month running mean sunspot numbers<sup>8</sup> (1750-1963) is given in Fig. 2c. Use of a longer time period in the analysis of the sunspot data is based on the assumption that all of the time series analysed here are stationary. Though spectral components determined using the MESA technique may be shifted somewhat in frequency<sup>7</sup>, similarities in the spectra are evident. Note that long-period components in the spectra for the cyclone data

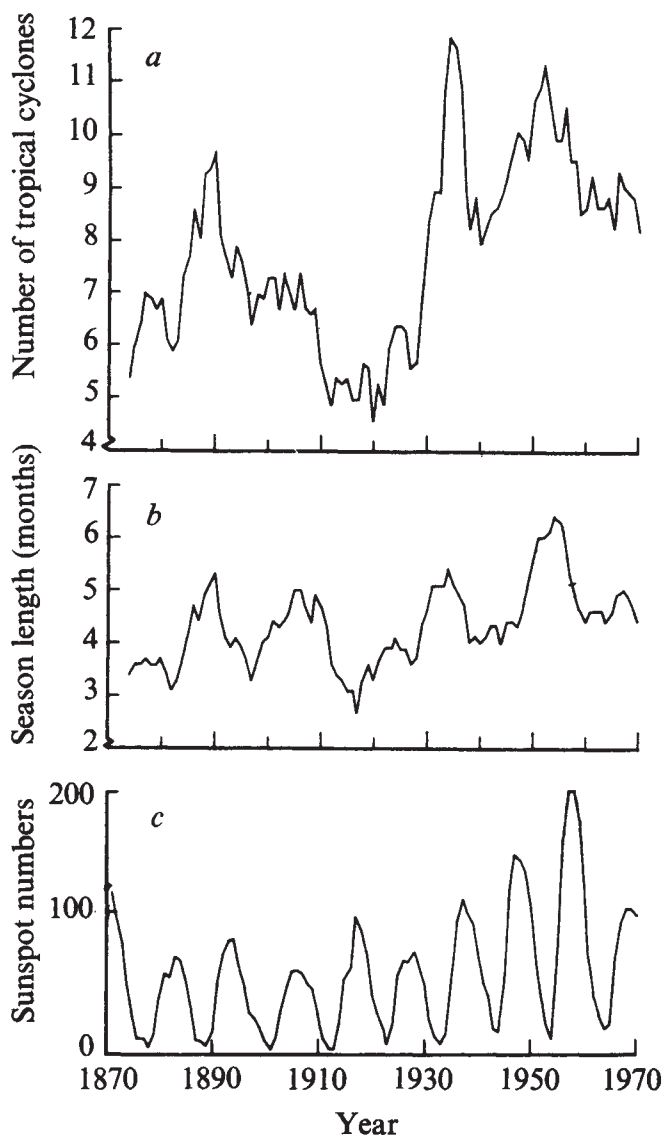


Fig. 1 a, 7-yr running mean number of Atlantic tropical cyclones; b, 7-yr running mean length of Atlantic tropical cyclone season; c, 12-month running mean sunspot numbers.

at 133 yr (Fig. 2a) and 154 yr (Fig. 2b) may represent ill-defined estimates for the 96 yr component found in the spectrum for the sunspot data. That these estimates are shifted to longer periods may result from the initial phase of the long period component in the window analysed or from the limited length of data available for analysis<sup>7</sup>. It is also possible that the data from 1871 to 1905 underestimate the number of cyclones which occurred each year as well as the annual lengths of the cyclone seasons, thereby introducing a long period bias into the data (after 1905, the use of radio aboard ships at sea greatly increased the quantity and quality of weather data acquired).

With respect to the 11-yr periodicities in tropical cyclone occurrence and length of season, it is interesting that some data for other meteorological phenomena exhibit this same periodicity. For example, Currie<sup>9</sup> observed a 10.6-yr periodicity in the spectra of surface air temperature data from North America, and concluded that solar activity has measurably affected this climatic element.

Although prominent spectral components at periods of 15 and 22 yr are found in the MESA spectra for the data on the length of the cyclone season, and appear only weakly in the sunspot spectrum, similarities in the MESA spectra



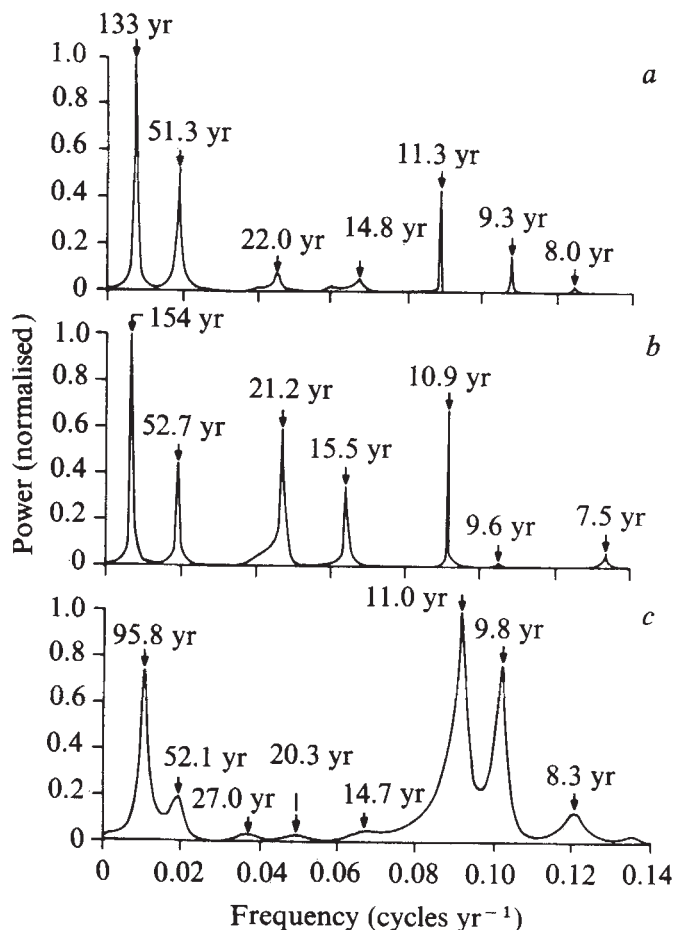


Fig. 2 Maximum entropy spectra for data shown in Fig. 1. a, Number of tropical cyclones; b, length of tropical cyclone season; c, sunspot numbers (spectra computed from data in the interval 1750–1963).

for the smoothed number of North Atlantic cyclones, the smoothed length of the cyclone season, and the 12-month running mean sunspot numbers suggest that a relationship exists between the solar cycle and Atlantic tropical cyclones. Whether the 22-yr periodicity found in the tropical cyclone data has its origin in the reversal of the magnetic fields associated with sunspots (the so-called “Hale double sunspot cycle”) is speculative. The possibility that a long period resonance exists in the Earth’s response to solar radiation must also be considered.

We offer no explanation as to how variations in the solar cycle influence the tropical cyclone phenomenon, and more generally, the climate.

We thank Dr R. Blandford and the referees for comments.

THEODORE J. COHEN  
ERTON I. SWEETSER

Teledyne Geotech,  
Alexandria, Virginia 22313

Received January 13; accepted May 30, 1975.

- <sup>1</sup> Landsberg, H. E., *J. geophys. Res.*, **65**, 1305–1307 (1960).
- <sup>2</sup> Cry, G. W., *Tropical Cyclones of the North Atlantic Ocean*, Tech. Paper 55 (Weather Bureau, US Department of Commerce, 1965).
- <sup>3</sup> Herbert, P. J., *North Atlantic Tropical Cyclones, 1973*, from NOAA Climatological Data, National Summary, 24 (NOAA, Boulder 1973).
- <sup>4</sup> Waldmeier, M., *The Sunspot Activity in the Years 1610–1960* (Technische Hochschule, Zurich, 1961).
- <sup>5</sup> Waldmeier, M., *Monthly Sunspot Bulletin from the Swiss Federal Observatory* (Zurich, 1960–1972).
- <sup>6</sup> Ulrych, T. J., *J. geophys. Res.*, **77**, 1396–1400 (1972).
- <sup>7</sup> Chen, W. Y., and Stegen, G. R., *J. geophys. Res.*, **79**, 3019–3022 (1974).
- <sup>8</sup> Cohen, T. J., and Lintz, P. R., *Nature*, **250**, 398–399 (1974).
- <sup>9</sup> Currie, R. G., *J. geophys. Res.*, **79**, 5657–5660 (1974).

## Odd and even numbered year summer temperature pulse in central England

METEOROLOGISTS and amateur weather observers with long memories have noted for some time that there seemed to be a preference for the hottest summers to occur during odd numbered years and the coolest during even numbered years during this century in south-eastern England. Many studies made during the past decade have supported the validity of such recollections for a much wider area. For example, Sutton<sup>1</sup> and Murray<sup>2</sup> have identified the existence of a biennial oscillation of the atmosphere and discussed its effects on the tropospheric circulation in both tropical and temperate latitudes. Davis<sup>3</sup> has found a high statistical significance in temperature differences between odd and even numbered years for an extensive area of western Europe.

The publication<sup>4</sup> of temperature observations for central England brings this long series up to date. The series, starting with 1721, has now been examined again to test whether the even-odd year cycle is peculiar to the summers of the present century, or whether it is indeed a long term feature of our climate. Data from years before 1721 have been excluded as they are less reliable. We have used the month of July; as the July temperature in England is highly correlated with sunshine and low rainfall it can be used as a good index of summer weather.

To remove the influence of longer period oscillations in the data, the anomalies in temperature for each year were obtained by using averages taken over decades. We have defined a tercile distribution of anomalies greater than + 0.4 °C, within ± 0.4 °C inclusive and less than – 0.4 °C. The distribution within these classes for even and odd years is shown in Table 1.

Table 1 Distribution of temperature anomalies in July (1721–1970)

|            | A > + 0.4 °C | B ± 0.4 °C | C < – 0.4 °C | Total |
|------------|--------------|------------|--------------|-------|
| Even years | 30           | 47         | 48           | 125   |
| Odd years  | 51           | 26         | 48           | 125   |

The  $\chi^2$  test shows that the distributions are significantly different to the 1% level. The differences show that in 21 even Julys there is a shift from above average to average compared with the odd Julys: the probability of an above average July in an odd year is 0.41 compared with 0.24 in an even year. The probability of a below average July is 0.38 for both odd and even years.

The period from 1911 to 1970 was examined separately. The distributions are shown in Table 2. The probability of having an

Table 2 Distribution of temperature anomalies in July (1911–1970)

|            | A > ± 0.4 °C | B ± 0.4 °C | C < – 0.4 °C |
|------------|--------------|------------|--------------|
| Even years | 5            | 11         | 14           |
| Odd years  | 14           | 9          | 7            |

above average July in an odd year has increased to 0.47 as against 0.17 for an even year. Likewise, the probability of a cool July in an even year has increased to 0.47.

The results show that in the whole period from 1721 to 1970 a highly significant biennial pulse is occurring in the July temperatures. The main characteristic of the pulse during the

past 60 yr is the occurrence of warm Julys in odd years and cool Julys in even years, though taken over the whole 250-yr period this characteristic is not so well marked.

A. H. GORDON  
N. C. WELLS

Department of Geophysics,  
University of Reading,  
Whiteknights Park,  
Reading, UK

Received May 5; accepted June 12, 1975.

- <sup>1</sup> Sutton, O. G., *Weather*, 17, 408 (1962).  
<sup>2</sup> Murray, R., *Weather*, 27, 161-169 (1972).  
<sup>3</sup> Davis, N. E., *Met. Mag., Lond.*, 96, 178-187 (1967).  
<sup>4</sup> Manley, G., *Q. Jl R. met. Soc.*, 100, 389-405 (1974).

## Brine at the bottom of the Great Bitter Lake as a result of closing the Suez Canal

DURING the process of clearing the Suez Canal and the Bitter Lakes from bombs and mines resulting from the 1967 and 1973 wars, an abnormal phenomenon was observed. Divers reported that diving was rather difficult through a layer extending about two metres above the bottom in the eastern part of the Great Bitter Lake (GBL). When this layer was reached through diving, any diver was forced to turn upwards. Moreover, sounding of depths by the use of sonic waves gave values which were less by about 2 m than when measured by weight and line.

A preliminary investigation of salinity was made on June 20, 1974, in the GBL. This showed that the salinity near the bottom was exceptionally great in many locations, especially in the eastern area of the lake. One of these locations was selected for detailed study. It was 16.5 m deep and located at a distance about 4 km east of the navigation channel. Water samples were collected at surface, 6, 12, 14 and 16 m depths, and temperatures at every depth were measured. Hydrogen sulphide was smelt distinctly in the samples collected at and below 14 m depth where several rotten fish were found. Bottom samples were sludgy and had a black colour.

Salinity was estimated by two different and independent methods after diluting the water samples with distilled water: determination of chlorinity by the Knudsen titration method<sup>1</sup>, in which chlorinity was converted to salinity using an empirical equation suggested by Morcos and Riley<sup>2</sup> for the Suez Canal region, and measurement of conductivity ratio. The two methods gave very close results.

Salinity and temperature values of the water column are given in Table 1; they show that, with increasing depth, the temperature variation is within the normal range while the increase of salinity is very rapid. A layer of brine (salinity more than 300‰) about 2 m thick exists above the bottom. This exceptionally high salinity had never been reported before.

The GBL was a dry basin before it was filled by water from the Mediterranean and Red Seas in November 1869. Its bottom is formed of multilayers of salt deposits separated by films of sand and gypsum. Early investigations<sup>3</sup> of the salinity of the Suez Canal and its lakes showed that even during the operation of filling the GBL with sea water, the highest salinity recorded was 168.6‰ (by evaporation) when water from the Mediterranean was covering the salt bed by only 1.4 m. Evidence based on observation<sup>4-6</sup> of salinity in 1924-25 and 1933-34, suggests that the southern part of the canal possessed higher salinity than the GBL itself. Observations during 1954 and 1955 indicate<sup>7</sup> that the maximum salinity in the Suez Canal

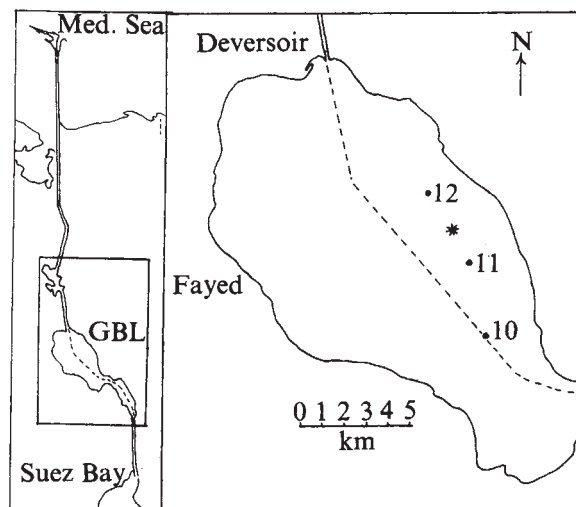


Fig. 1 Map of the Great Bitter Lake showing the locations of stations 10, 11 and 12 occupied by El-Sharkawy<sup>8</sup> in April 1967 and the position (\*) where the samples were collected in June 1974.

region (48.4‰) was reached in late summer in the southern canal but not over the salt bed as might be expected. El-Sharkawy<sup>8</sup> carried out observations of salinity in the Bitter Lakes for one year (April 1966-April 1967) and the salinity in GBL did not exceed 49‰. His last cruise (April 1967) is of great interest since it was made only two months before the closing of the Suez Canal that June. The salinity-depth profile of the three stations 10, 11, 12, which were occupied in the eastern part of the GBL (Fig. 1) is reproduced in Table 2. His deepest samples were obtained at only 12 m depth, but the salinity was increasing gently with depth and did not show any indication of an abrupt increase. By contrast, my results (Table 1) show unusually high salinity at 6 m and approach saturation at 14 m. Comparison between Tables 1 and 2 shows that my surface salinity is even higher than that obtained at 12 m on the 1967 cruise. This clearly indicates that a rather abnormal phenomenon occurred in the GBL during the period of closure of the Suez Canal.

In normal conditions the salinity of the GBL is influenced by the time of residence of water over the bottom salt deposits, the exchange of water with the southern and northern parts of the canal, and evaporation and meteorological conditions in the region. But in the special situation after the Suez Canal was closed, the first two factors were the principal causes of the exceptionally high salinity of the GBL and the formation of a brine layer near its bottom. The rate of salinity increase is directly proportional to the time of residence of water over the salt bed and inversely proportional to the rate of water flow through the Suez Canal. This was confirmed by observations<sup>7</sup> which show that during June-July, when the northward flow in Suez Canal becomes very slow and thus the time of residence becomes relatively longer, the salinity of the GBL reaches its maximum value of the year.

Table 1 Variation of salinity and temperature with depth at a station in the GBL (Fig. 1) occupied on June 20, 1974

| Depth (m) | Salinity (‰) | Temperature (°C) |
|-----------|--------------|------------------|
| 0         | 48.50        | 30.32            |
| 6         | 63.60        | 28.41            |
| 12        | 151.29       | 26.64            |
| 14        | 311.17       | 25.90            |
| 16        | 314.93       | 25.63            |

**Table 2** Salinity-depth profile at stations 10, 11 and 12 in the GBL observed on April 5, 1967 (ref. 8)

| Depth (m) | 10    | Salinity ‰ | 11    | 12 |
|-----------|-------|------------|-------|----|
| 0         | 43.23 | 43.41      | 43.63 |    |
| 3         | 43.48 | 43.75      | 43.93 |    |
| 6         | 43.88 | 43.93      | 44.02 |    |
| 9         | 43.97 | 44.04      | —     |    |
| 12        | 44.35 | —          | —     |    |

After June 1967 the Suez Canal was closed to navigation and many obstacles and wrecks were blocking the waterway. Moreover, during the period from October 1973 to June 1974 the Canal was completely blocked by an earth dam at Deversoir where the Canal enters the northern part of the GBL. So the normal exchange of water between the Red and Mediterranean Seas through the Suez Canal was very much reduced and eventually stopped. This exchange was the chief cause for the gradual decrease of the salinity of the GBL from 168.8‰, during its filling process, to an average of 46‰ in April 1967. As the water passes over the salt bed, a considerable quantity of salt goes into solution and is eventually carried away by the water flow to the adjacent seas. In the new situation the GBL was almost isolated and its water became stationary over the salt bed allowing the salt dissolution to continue for a time long enough to bring the bottom water very close to its saturation point. The strong stratification and the great stability of the water column observed in June 1974 (Table 1) overcame the effects of wind stirring and evaporation.

The dense water (density about  $1.2 \text{ g ml}^{-1}$ ) near the bottom of the GBL acted as a barrier to divers, who found it difficult to penetrate. The black mud on the top of the salt bed was also observed by the Cambridge Expedition<sup>4</sup> in 1924 and in 1966–67 by El-Sharkawy<sup>8</sup>.

It seems likely that as soon as the exchange between the canal and the adjacent seas returns to its normal magnitude, the brine layer will disappear and the salinity at all depths will decrease. I estimate that there will be a sharp drop in the salinity of the brine layer in the first few years followed by a gradual slow decrease afterwards. This assumption is based on the fact that after opening the Suez Canal in 1869 the average annual decrease of the salinity of the GBL was 0.36‰ in the period from 1872 to 1898, became 0.27‰ from 1898 to 1924 and then 0.13‰ from 1924 to 1966 (ref. 8). Although there is no record of the rate of decrease of the salinity in the period from June 1869, during the filling process when the salinity was 168.8‰, to 1872, this rate can be approximately estimated. Taking the salinity in 1966 as 46‰ and the salinity decrease in the period from 1872 through 1966 as 21.84‰, it follows that the salinity in 1872 was of the order of 67.8‰. This means that the annual decrease of salinity in the first two years after opening the canal might have been very sharp.

I strongly suggest that a thorough investigation of the physical, chemical and biological characteristics of the Bitter Lakes and Suez Canal could be made during the present transitional period.

AMIN H. MESHAL\*

*Institute of Oceanography and Fisheries,  
Alexandria, Egypt*

Received March 12; accepted May 29, 1975.

\*Present Address: Air-Sea Interaction, Department of the Environment (AOL), Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada B2Y 4A2.

<sup>1</sup> Knudsen Hydrographical Tables (G. E. C. Gad, Copenhagen, 1901).

<sup>2</sup> Morcos, S. A., and Riley, J. P., *Deep Sea Res.*, 13, 741–749 (1966).

<sup>3</sup> Morcos, S. A., *Proc. R. Soc. Edinb.*, B72, 449–458 (1972).

<sup>4</sup> Fox, M. H., *Trans. zool. Soc. Lond.*, 22, 1–64 (1926).

<sup>5</sup> Wust, G., *Naturwissenschaften*, 22, 446–450 (1934).

<sup>6</sup> Faouzi, H., *Ann. Centre Univ. Hediter.*, 5, 23–30 (1951).

<sup>7</sup> Morcos, S. A., and Messieh, S. N., *Limnol. Oceanogr.*, 18, 121–130 (1973).

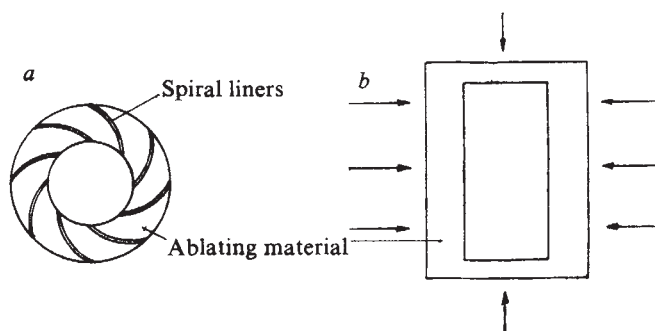
<sup>8</sup> El-Sharkawy, S. M., thesis, Univ. Cairo (1969).

## Confinement of thermonuclear microexplosions in imploding vortices

PRESENT efforts towards laser induced fusion envision spherical pellets consisting of the thermonuclear material in the centre, surrounded by a shell of some inert pusher material and an outer ablator shell. The same principles for the pellet design can be used if the implosion process is induced by a relativistic electron beam or intense ion beam (ref. 1, papers presented to Fifth IAEA Conference, Tokyo, 1974 and J. W. Shearer, unpublished). At least one other design of a non-spherical fuel pellet has been published<sup>2</sup>. This is non-symmetric and would have the advantage of using just one laser beam for target bombardment. But in all of these designs there remains one principal difficulty: laser, electron or ion beams with a sufficiently large total energy output for high density pellet compression and efficient thermonuclear burn have a comparatively long pulse length. Therefore, in order to use these beams for the required high density pellet compression rather large aspect ratios  $r/\Delta r$  ( $r$ =pellet radius,  $\Delta r$ =thickness of pusher and ablator material) are dictated. These may result in rapidly growing Taylor-type instabilities. The growth rate for these instabilities could in principle be reduced below dangerous levels by making the pellet and implosion process highly symmetrical. But, since the symmetry requirements are extreme this may be difficult to achieve. The growth rate for the Taylor instabilities could also be reduced by smaller aspect ratios, but smaller aspect ratios need shorter and precisely shaped pulses, which again are difficult to achieve.

I propose here an alternative pellet design which assures Taylor-stable implosion at high aspect ratios. Figure 1 shows the new pellet design in two cross sections. The pellet has the form of a circular cylinder with cylindrical liners of spiral shape embedded in the pusher and ablator material. The arrows indicate the beams radially and axially bombarding the pellet. In addition to the radial beams, two axial beams are required to compress the thermonuclear material within the pellet axially. The presence of the spiral liners, which are made of some heavy inert material, results in an azimuthal velocity component for the ablated material. If the liners have the form of logarithmic spirals, the ratio of the radial to the azimuthal ablation velocity is constant during the ablation process. As a result of the radial ablation the pellet material is imploded. But the implosion process is here governed not only by the conservation of radial linear momentum but also by the conservation of angular momentum. This results in a collapsing vortex similar to a tornado funnel. The azimuthal motion in the collapsing vortex will give rise to two effects. First, it will stop

Fig. 1 The new pellet design. a, Plan; b, elevation.





the collapse at a minimum radius of the vortex core at the moment the centrifugal forces become equal to the implosion stagnation force; second, the rapid rise of the centrifugal force during the collapse will make the implosion process Taylor-stable (as in the case of the formation of a tornado funnel). The ratio of collapse ( $r_0/r_1$ , where  $r_0$  is the initial radius of the vortex core and  $r_1$  its final minimum radius) can be predetermined by a proper choice of ratio  $v_r^{(0)}/v_\phi^{(0)}$  for the initial ratio of the radial to the azimuthal implosion velocity and thus the slope of the liner spirals. At maximum collapse  $v_r = v_r^{(1)} = 0$  and  $v_\phi = v_\phi^{(1)}$ .

Conservation of energy and angular momentum then require that

$$(v_\phi^{(1)})^2 = (v_r^{(0)})^2 + (v_\phi^{(0)})^2 \quad (1)$$

$$r_1 v_\phi^{(1)} = r_0 v_\phi^{(0)} \quad (2)$$

From this one obtains

$$v_r^{(0)}/v_\phi^{(0)} = [(r_0/r_1)^2 - 1]^{1/2} \approx r_0/r_1, \quad r_0 \gg r_1 \quad (3)$$

If one assumes a tenfold compression in radius this yields  $v_r^{(0)}/v_\phi^{(0)} \approx 10$ .

An inertial force directed towards decreasing density results in a Taylor-unstable situation and one directed towards increasing density results in Taylor-stable fluid flow. For the case in question the inertial force resulting from the radial deceleration

$$a_1 \approx (v_r^{(0)})^2/r_0 \quad (4)$$

is destabilising, whereas the centrifugal force resulting from the acceleration

$$a_2 \approx (v_\phi^{(1)})^2/r_1 \quad (5)$$

is stabilising. Stability is guaranteed if  $a_2/a_1 \gg 1$ . From equations (2) and (3) this condition leads to

$$a_2/a_1 \approx r_0/r_1 \gg 1 \quad (6)$$

which in my chosen example,  $r_0/r_1 = 10$ , is well satisfied.

The proposed scheme of a collapsing vortex may be used also for the stable confinement of a microexplosion induced by a high density gas-embedded pinch. Because of the longer time scale involved in such a case, the ablator material may be made out of high explosive material which is radially detonated, eliminating the need for laser or other intense beams to affect the ablation-induced implosion and compression.

F. WINTERBERG

Desert Research Institute,  
University of Nevada System,  
Reno, Nevada 89507

Received May 9; accepted May 30, 1975.

<sup>1</sup> Winterberg, F., *Nature*, **251**, 44 (1974); *Plasma Physics*, **17**, 69 (1975).

<sup>2</sup> McCall, G. H., and Morse, R. L., *Laser Focus*, **12**, 40 (1974).

## Formation of blisters in molybdenum bombarded with helium

THE formation of surface blisters on metals bombarded with low energy inert gas ions was first observed by Primak<sup>1</sup> but only recently, when the potential importance of blistering in the first wall of a fusion reactor became recognised, has much further attention been paid to the phenomenon<sup>2,3</sup>. Most of this work has used scanning electron microscopy to

examine the blistered surface although considerable information has also been obtained from gas release measurements both during bombardment and in post-bombardment anneals. Transmission electron microscopy has been used to a lesser extent but nevertheless has yielded important information on the substructure within the layer where the gas atoms came to rest—normally some hundreds of Ångströms from the metal surface. For example, several workers have shown that gas bubbles form in thin foils irradiated with low energy (<300 keV) helium or argon ions<sup>4-6</sup>. One interesting fact (D. J. Mazey, unpublished) is that over a wide temperature range from 600 °C down to 20 °C, 36 keV helium bombardment of molybdenum produces a fine distribution of helium bubbles crystallising on to a body-centred-cubic (b.c.c.) lattice analogous to the void lattice<sup>4,7</sup>. Here I describe an extension of transmission microscopy to view the blisters themselves and suggest some details of the blister formation mechanism.

Molybdenum samples were irradiated at room temperature with  $5 \times 10^{17}$  18-keV helium ions per cm<sup>2</sup>, these ions having a projected range of approximately 630 Å. Following bombardment the samples were electropolished using a backthinning technique to protect the front damaged surface so that this surface was contained in the final electron-transparent thin section. Both a Philips EM 300 100 keV electron microscope and the Harwell AEI High Voltage Microscope were used for specimen examination. On either microscope the presence of the blisters (Fig. 1),

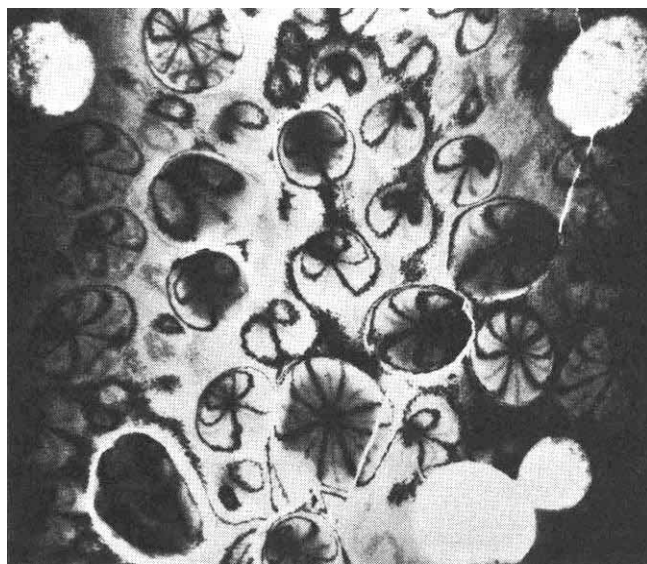


Fig. 1 800-keV electron micrograph of blisters in helium-bombarded molybdenum. The blisters have diameters between 1 and 1.5  $\mu\text{m}$ .

was made very obvious by the bend contours associated with their dome-shaped profile. The contour patterns seem to be a good example of the type used by Steeds *et al.*<sup>8</sup> for real space crystallography; it was simple to index each contour and use the poles (Fig. 2) to measure the blister radius of curvature. On a finer scale the damage structure consisted of a high density of dislocations—thus making the bend contours considerably less sharp than in a perfect crystal—and a very high concentration of small helium bubbles, radius 15–20 Å. These bubbles were in both the unblistered areas and the blister covers.

McCracken<sup>9</sup> has briefly outlined the process of blister formation as the coalescence of small helium bubbles to form a large high pressure bubble sufficient to deform the surface. This seems to be consistent with the experimental

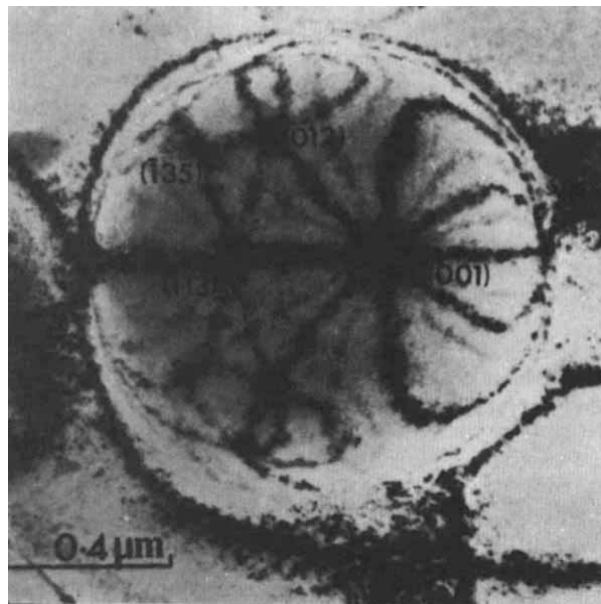


Fig. 2 A more detailed micrograph of blister bend contours with the main poles indexed.

observations but any detailed mechanism besides describing the formation of the blisters from the small bubbles must also explain at least two important results in the blistering work:

- (1) Both scanning electron microscopy and gas release results have demonstrated that a relatively sharp threshold dose exists for blister formation.
- (2) At high temperatures the bombarded surface displays a 'pinhole' structure rather than blisters<sup>9,10</sup>.

The possible mechanism suggested here is based on the assumption that the coalescence of growing bubbles (at temperatures below that at which bubbles can thermally migrate) only becomes rapid when the bubbles are essentially touching—in other words when the volume swelling due to the bubbles reaches a critical value. In the case of the bubbles being on a perfect b.c.c. lattice, it is easy to show that the volume swelling ( $\Delta V/V$ ) would be 68% but in the random array the situation is a little less certain. But if we proceed on this basis, the reason for the existence of a critical dose is evident and is governed by the maximum volume swelling that can be supported in the peak of the helium stopping distribution. The interesting point is that because the threshold condition is a volume one, then coalescence without any decrease in volume—the likely situation unless the bubble can quickly acquire excess interstitials—will not relieve the tendency for further coalescence. So, on this model the coalescence, once started, becomes a runaway process. From the point of view of blistering the important thing is that the coalescence of two gas bubbles at constant volume must increase the excess internal pressure. For example, if we take two equal equilibrium gas bubbles, radius  $r$ , then the internal pressure,  $p$ , is given by  $p = 2\gamma/r$  where  $\gamma$  is the surface energy. On coalescing, the new equilibrium gas pressure will be lower by a factor of  $2^{1/3}$ , giving the bubble an excess internal pressure 26% above the equilibrium. As further coalescence takes place, this value will rapidly increase until enough excess pressure is built up to deform the metal surface and create a blister. For the small gas bubbles observed, the equilibrium gas pressures are in the region of  $10^4$  atmospheres.

At high temperatures the availability of thermal vacancies becomes important<sup>11</sup> between 0.4 and  $0.5T_m$ . It is therefore

possible to define a temperature where the coalesced bubbles immediately collect enough vacancies to relieve their excess internal pressure and regain equilibrium. In this way, not enough pressure can build up to deform the metal surface and blisters will not be formed. Instead the bubbles grow until they cut the surface thus producing the observed 'pinhole' effect.

One important prediction of this model is that in the fusion reactor first wall, which will have a spectrum of ion energies, the integrated dose necessary to build up the critical helium concentrations at any particular depth will be far greater than those found experimentally with mono-energetic beams. Such results would overestimate predicted first wall surface erosion rates and thus the detrimental effects of blistering could be less than anticipated.

I thank colleagues in the Metallurgy and Theoretical Divisions at Harwell and Drs G. M. McCracken and K. Erents from Culham for discussions.

J. H. EVANS

Metallurgy Division,  
Atomic Energy Research Establishment,  
Harwell, UK

Received May 14; accepted June 19, 1975.

- <sup>1</sup> Primak, W., *J. appl. Phys.*, **34**, 3630 (1963).
- <sup>2</sup> *Applications of Ion Beams to Metals* (edit. by Picraux, S. T., EerNisse, E. P., and Vook, F. L.), (Plenum, New York, 1974).
- <sup>3</sup> *Proc. Conf. on Surface Effects in Controlled Fusion Devices*, *J. nucl. Mat.*, **53** (1974).
- <sup>4</sup> Sass, S. L., and Eyre, B. L., *Phil. Mag.*, **27**, 1447 (1973).
- <sup>5</sup> Thomas, G. J., and Bauer, W., in *Applications of Ion Beams to Metals* (edit. by Picraux, S. T., EerNisse, E. P., and Vook, F. L.), 533 (Plenum, New York, 1974).
- <sup>6</sup> Hertel, B., Diehl, J., Gotthardt, R., and Sultze, H., *ibid.*, 507.
- <sup>7</sup> Evans, J. H., *Nature*, **229**, 403 (1971).
- <sup>8</sup> Steeds, J. W., Tatlock, G. J., and Hampson, J., *Nature*, **241**, 435 (1973).
- <sup>9</sup> McCracken, G. M., *Rep. Progr. Phys.*, **38**, 241 (1975).
- <sup>10</sup> Erents, S. K., and McCracken, G. M., *Rad. Effects*, **18**, 191 (1973).
- <sup>11</sup> Martin, D. G., *Culham Report*, R-103 (UKAEA, 1970).

## A novel method for the calculation of energies of activation for gas reactions

A GENERAL method for the rapid, accurate calculation of activation energies for gas reactions would clearly be of great value. To date, full *ab initio* quantum mechanical calculations of reaction coordinates and potential energy surfaces have been carried out for only the simplest systems and extensions to larger ones are still some way off<sup>1</sup>. On the other hand, semi-empirical approaches<sup>2</sup> which could perhaps deal with larger species require a good deal of accessory experimental information.

We suggest here a simple new approach, based on self-consistent molecular orbital calculations of the Pople-Segal-Santry type<sup>3,4</sup>. This leads first to the heat of activation,  $\Delta H^\ddagger$  for a reaction and through this to a value for the energy of activation ( $\Delta E^\ddagger$ ).

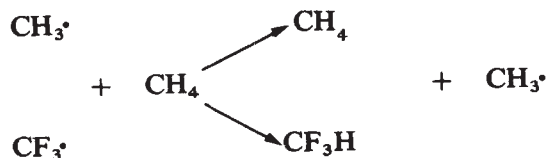
The use of the 'bond index' has been derived and illustrated previously<sup>5,6</sup>. This quantity stems from the density matrix which is, in turn, readily obtainable from a self-consistent field molecular calculation. It is found, for example, that, in the case of  $\text{CH}_4$ , the C-H bond index is 0.987, and the C-C bond indices in the series  $\text{C}_2\text{H}_6$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_2\text{H}_2$  are 1.023, 2.032, 2.989. These indices clearly correspond to the chemist's concept of a 'single' bond or a 'double' bond and, on this basis, we suggest that the bond indices can be used, together with experimental heats of atomisation in the standard state, to generate an improved set of standard energy terms for a variety of bonds. These will then allow prediction of the heats of formation for molecules containing them. A particular improvement over the present bond energy concept is that the bond index reflects slight changes in intrinsic bond strength with the environment of the bond in question, further details of which will be published elsewhere (P.G.P.). But an important extension arises because bond indices can be calculated for any system, and can thus



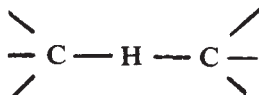
afford, in principle, the heat of atomisation for that system in the standard state.

So the method should be applicable to a species undergoing reaction. By constructing a grid of points on a surface each corresponding to a particular geometry we can follow a reaction path over a saddle. Because of the way in which the bond energy terms are initially defined, we thus obtain the heat of activation,  $\Delta H^\ddagger$ , in the standard state. Calculation of the energy of activation,  $\Delta E^\ddagger$ , follows in a standard way<sup>7</sup>.

We here apply the technique to two related hydrogen-transfer reactions, namely, hydrogen abstraction from methane by the methyl and the trifluoromethyl radical,



We assumed that all positions on the reaction coordinate have  $C_{3v}$  symmetry, thus,



We further assumed that, in the reaction between methyl and methane, the starting and resultant methyl radicals undergo linear changes in the planar  $\longleftrightarrow$  pyramidal angles. The  $\text{CF}_3\cdot$  radical was assumed pyramidal throughout. At the starting point the reactants were separated by 0.5 nm. An energy search was carried out around the transition state in each case and the reaction coordinates were constructed. The activation energies, corrected for specific heat changes on formation of the transition

**Table 1** Energies of activation for the reactions  $\text{CH}_3\cdot$  or  $\text{CF}_3\cdot + \text{CH}_4 \rightarrow \text{CH}_4$  or  $\text{CF}_3\text{H} + \text{CH}_3\cdot$

| Reactant           | $\Delta E^\ddagger$ (kJ mol <sup>-1</sup> ) |            |
|--------------------|---------------------------------------------|------------|
|                    | Observed                                    | Calculated |
| $\text{CH}_3\cdot$ | 59.0, 59.8, 62.3*                           | 56.2       |
| $\text{CF}_3\cdot$ | 46.0†                                       | 49.4       |

\*Tables of Bimolecular Gas Reactions, A. F. Trotman-Dickenson and G. S. Milne.

†As for \*; recommended value.

state, are given in Table 1. These are compared with the experimental values by assuming a temperature in the middle of the experimental range. The agreement is very encouraging and we are now investigating the use of the approach on a series of other gas reactions.

THOMAS N. BELL

Department of Chemistry,  
Simon Fraser University,  
Burnaby 2, British Columbia, Canada

PETER G. PERKINS

Department of Pure and Applied Chemistry,  
University of Strathclyde,  
Glasgow G1 1XL, UK

Received May 7; accepted May 22, 1975.

<sup>5</sup> Armstrong, D. R., Perkins, P. G., and Stewart, J. J. P., *J. chem. Soc., Dalton Trans.*, 838 (1973).

<sup>6</sup> Armstrong, D. R., Perkins, P. G., and Stewart, J. J. P., *J. chem. Soc., Dalton Trans.*, 2273 (1973).

<sup>7</sup> Glasstone, S., Laidler, K. J., and Eyring, H., *The Theory of Rate Processes*, ch. IV (McGraw-Hill, New York and London, 1941).

## Stereochemistry of some steranes from geological sources

THE steranes present in crude oils and sedimentary rocks are believed to be derived from the sterols of ancient organisms. Their precise structure and stereochemistry may give important clues about the nature of the original biological materials and about the geochemical processes which they have undergone. Examination of synthetic steranes<sup>1,2</sup> has led us to question the adequacy of reported<sup>3-6</sup> identifications, using only gas-liquid chromatography (GC) and mass spectroscopy (MS), of steranes such as 5 $\alpha$ -cholestane, 5 $\alpha$ -ergostane, and 5 $\alpha$ -stigmastane (= 5 $\alpha$ -sitostane) in geological samples. The last two, in particular, seem incompletely identified, since the configuration at C-24 has been ignored (although it is of phylogenetic importance in modern organisms<sup>7,8</sup>), and since we find that epimers at C-24, that is II and III, IV and V, are indistinguishable using GC and MS. The use of the names 5 $\alpha$ -ergostane and 5 $\alpha$ -stigmastane in geochemical work both for steranes of undetermined configuration at C-24 and for synthetic samples of defined configuration is confusing. In the absence of clear IUPAC/IUB rules, we have adopted the names shown in Fig. 1.

The structure and absolute stereochemistry of many steranes can, however, be defined<sup>1,2</sup>, after isolation, by the additional use of optical rotatory dispersion (ORD) and high-resolution (100 or 220 MHz) proton magnetic resonance (PMR) spectroscopy. Preliminary results on the application of these methods to steranes from two crude oils and from Green River Shale are reported here.

A crude oil of unknown age from Rozel Point, Utah<sup>9</sup>, was distilled, chromatographed on silicic acid and alumina, and treated with urea to give a sterane-rich fraction. Examination using GC and MS revealed a large number of  $\text{C}_n\text{H}_{2n-6}$  ( $n = 26-30$ ) components with sterane-type fragmentation patterns.

Further separation was achieved by thiourea adduction and preparative GC. A component having the retention time of 5 $\alpha$ -cholestane was recrystallised from propanol and confirmed as 5 $\alpha$ -cholestane (I) by melting point (80–80.5 °C), mixed melting point, MS, PMR, and ORD. A second recrystallised isolate (melting point 80.5–81 °C), indistinguishable from 5 $\alpha$ -ergostane using GC and MS, was revealed as a 1:1 mixture of 5 $\alpha$ -campestan (II) and 5 $\alpha$ -ergostane (III) using PMR and ORD. These two steranes were separated on alumina (2.8 m  $\times$  5 mm column eluted with pentane; method to be published) and the identity of each was confirmed using PMR spectroscopy. The presence in the thiourea adduct of (24R)- and (24S)-4 $\alpha$ , 24-dimethyl-5 $\alpha$ -cholestanes in a similar ratio was also demonstrated. A component having the GC and MS properties of 5 $\alpha$ -stigmastane was isolated from the thiourea non-adducted material and examined without recrystallisation; PMR analysis indicated a 1:1 mixture of 5 $\alpha$ -stigmastane (IV) and 5 $\alpha$ -poriferastane (V), consistent with the ORD spectrum.

Similar fractionation of a crude oil of Upper Miocene origin from Huntington Beach, California<sup>10,11</sup> also yielded a 1:1 mixture of the epimers II and III. By contrast, the corresponding fraction isolated from a sample of Green River Shale (Eocene) from the Rifle Mine, Colorado, proved to be a 25:75 mixture of epimers II and III, together with a small quantity of an unidentified third component, whereas the major  $\text{C}_{29}$ -sterane fraction proved to be a 30:70 mixture of IV and V. Although comparisons between data from different samples of the vast

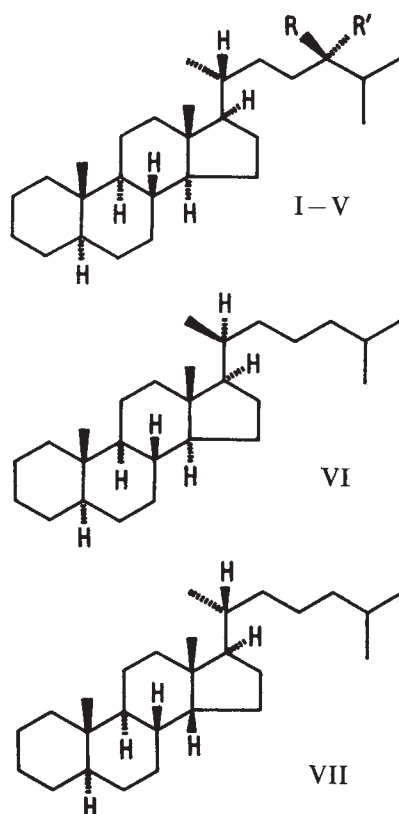
<sup>1</sup> Laidler, K. J., *Theories of Chemical Reaction Rates*, 8 (McGraw Hill, New York and London, 1969).

<sup>2</sup> Johnston, H. S., *Gas-Phase Reaction Rate Theory*, 177–183 (Ronald Press, New York, 1966).

<sup>3</sup> Pople, J. A., Santry, D. P., and Segal, G. A., *J. chem. Phys.*, 43, 5129 (1965).

<sup>4</sup> Armstrong, D. R., Perkins, P. G., and Stewart, J. J. P., *J. chem. Soc. A.*, 3654 (1971).





**Fig. 1** Structural formulae of steranes isolated from geological sources. I: R = R' = H; 5α-cholestane. II: R = methyl group; R' = H; 5α-campestane (24R). III: R = H; R' = methyl group; 5α-ergostane (24S). IV: R = ethyl group; R' = H; 5α-stigmastane (24R). V: R = H; R' = ethyl group; 5α-poriferastane (24S).

Green River Shale formation require caution, our result for the C<sub>28</sub>-sterane is compatible with the published <sup>13</sup>C nuclear magnetic resonance spectrum<sup>12</sup> of Green River Shale "5α-ergostane", which shows deviations from the reference spectrum in the regions (161 and 174 p.p.m. from CS<sub>2</sub>) where we find 5α-campestane (II) to differ from 5α-ergostane (III). This is consistent with the presence of a significant proportion of II in that sample.

At least two stereoisomers of 5α-cholestane are present in oil from Rozel Point. (20S)-5α-cholestane (VI) was isolated from the thiourea non-adduct and identified by comparison (GC, MS, PMR, ORD) with an authentic sample<sup>2</sup>; its concentration in the crude oil seems to be comparable with that of 5α-cholestane. A minor C-27 component of the thiourea adduct, homogeneous by GC analysis on several stationary phases, had the retention time of 5α,14β-cholestane (VIII), (ref. 2) and a similar mass spectrum, although the optical activity was considerably lower than expected. The PMR spectrum was interpreted as that of a 40:60 mixture of VII and an isomer in which the 18-CH<sub>3</sub> and 21-CH<sub>3</sub> signals are shifted upfield (δ 1.05 and 0.865, respectively, in C<sub>6</sub>D<sub>6</sub>). A possible structure for the latter is (20S)-5α,14β-cholestane, and the synthesis of a reference sample is in hand.

Although investigation of these geological samples is incomplete (for example, the identified steranes account for <20% weight for weight of all sterane-like hydrocarbons in Rozel Point crude oil), several interesting points are raised. The preponderance in Green River Shale of one epimer of each of the 24-alkyl-5α-cholestanes suggests that in relatively mild conditions of diagenesis the stereochemistry of precursor sterols is at least partly preserved in the hydrocarbon products; similarly, pristane from this source largely retains the stereochemistry of its putative precursor<sup>13</sup>. The predominant (24S)

stereochemistry may, in addition, point to possible source organisms such as algae<sup>8,14</sup>. The phylogenetic significance of the stereochemistry at C-24 requires further study, however, especially as it is now known that some higher plants produce 24-methylsterols of both (R) and (S) configuration (Mulheirn, L. J., unpublished). Our results also suggest that equilibration at C-20 and C-24 may prove characteristic of petroleum steranes, reflecting the more severe conditions required for petroleum formation. No stereochemical analyses of phytane or pristane from crude oils have been reported, although it may be relevant that farnesane from New Ulm (Texas) crude exhibits complete randomisation of stereochemistry at its two centres of asymmetry (J. W. Cornforth and J. R. Maxwell, personal communication). The occurrence of minor amounts of a 14β-sterane in Rozel Point oil may also be attributed to an equilibration process, the scanty available evidence<sup>15</sup> suggesting that this configuration is the less stable. No natural sterols with a 14β-hydrogen are known.

Finally, the data demonstrate for the first time that the isolated steranes possess the same absolute stereochemistry as that of the corresponding hydrocarbons synthesised from the sterols of modern organisms. No optical rotations have previously been reported for individual steranes from geological samples.

We thank Professor J. W. Cornforth for initiating this work; Drs A. Hood and P. R. Mommessin (Shell Development Company, Houston, Texas) for arranging the supply of the oils and shale; our colleagues at Shell Research Ltd, Thornton Research Centre, Cheshire for preliminary processing of the oils; and Mrs D. Davis, Mrs P. R. Jennings and Mrs S. A. Terry for technical assistance.

L. J. MULHEIRN  
G. RYBACK

Shell Research Limited,  
Milstead Laboratory,  
Sittingbourne Research Centre,  
Sittingbourne, Kent, ME9 8AG, UK

Received April 23; accepted June 11, 1975.

- Mulheirn, L. J., *Tetrahedron Lett.*, 3175-3178 (1973).
- Mulheirn, L. J., and Ryback, G., *J. chem. Soc. Chem. Comm.*, 886-887 (1974).
- Hills, I. R., Smith, G. W., and Whitehead, E. V., *J. Instn Petrol.*, 56, 127-137 (1970).
- Ikan, R., and Bortinger, A., *Israel J. Chem.*, 9, 679-682 (1971).
- Gallegos, E. J., *Analyt. Chem.*, 43, 1151-1160 (1971).
- Maxwell, J. R., Pillinger, C. T., and Eglinton, G., *Q. Rev. chem. Soc.*, 25, 571-628 (1971).
- Goad, L. J., and Goodwin, T. W., *Prog. Phytochem.*, 3, 113-198 (1972).
- Nes, W. R., *Lipids*, 9, 596-612 (1974).
- Eardley, A. J., *Spec. Stud. Utah geol. mineral. Surv.*, 5 (1963).
- Hoering, T. C., *Yb. Carnegie Instn Wash.*, 303-307 (1969).
- Philippi, G. T., *Geochim. cosmochim. Acta*, 29, 1021-1049 (1965).
- Balogh, B., Wilson, D. M., and Burlingame, A. L., *Nature*, 233, 261-263 (1971).
- Maxwell, J. R., Cox, R. E., Ackman, R. G., and Hooper, S. N., in *Advances in Organic Geochemistry 1971* (edit. by von Gaertner, H. R., and Wehner, H.), 277-291 (Pergamon, Oxford, 1972).
- Bradley, W. H., *Bull. geol. Soc. Am.*, 81, 985-1000 (1970).
- Van Horn, A. R., and Djerassi, C., *J. Am. chem. Soc.*, 89, 651-664 (1967).

## Influence of interannual climatic fluctuations on biological systems

GROWTH of conifers in western North America and the distribution of albacore tuna (*Thunnus alalunga*) along the west coast of North America are linked by large scale atmospheric flow patterns which are influenced by air-sea interaction processes over the eastern North Pacific. Although the systems respond to their respective environments during different times of the year there is strong evidence that they are reacting to the same climatic fluctuations.

Ring widths of conifers from a variety of semi-arid sites in western North America reflect climatic variations. The spatial anomaly patterns of tree growth have been related to large scale anomaly patterns of the general atmospheric circulation which, in turn, are related to anomaly patterns

of sea-surface temperature (see, for example, refs 1 and 2).

Seasonal migrations of albacore tuna into and out of the coastal North American fishery and the population distribution along the coast are related to changes in the thermal structure of the eastern North Pacific which, in turn, are related to air-sea interaction events<sup>3</sup>.

Although it would be desirable to know the yearly variations in the distribution of albacore abundance or the exact number of individuals in the population, the best source of available data is landing statistics compiled for various regions along the North American coast (J. A. Renner, personal communication). These data indicate albacore availability, or accessibility to the efforts of a fishery. A complex situation probably exists where fluctuations in landings result both from environmental influences affecting albacore migration routes (causing yearly variations in their geographical distribution along the coast) and from weather conditions affecting the location and success of the fishermen and/or the behaviour of the fish once they are distributed.

As an indicator of variations in albacore population distribution, the landings reported north of San Francisco were expressed as a percentage of those reported along the entire coast. This assumes the fishing effort is spread along the entire coast throughout each fishing season. Although a complete effort analysis has not been made, boats were operating consistently along the entire coast throughout the analysis period.

To determine whether spatial patterns of tree growth

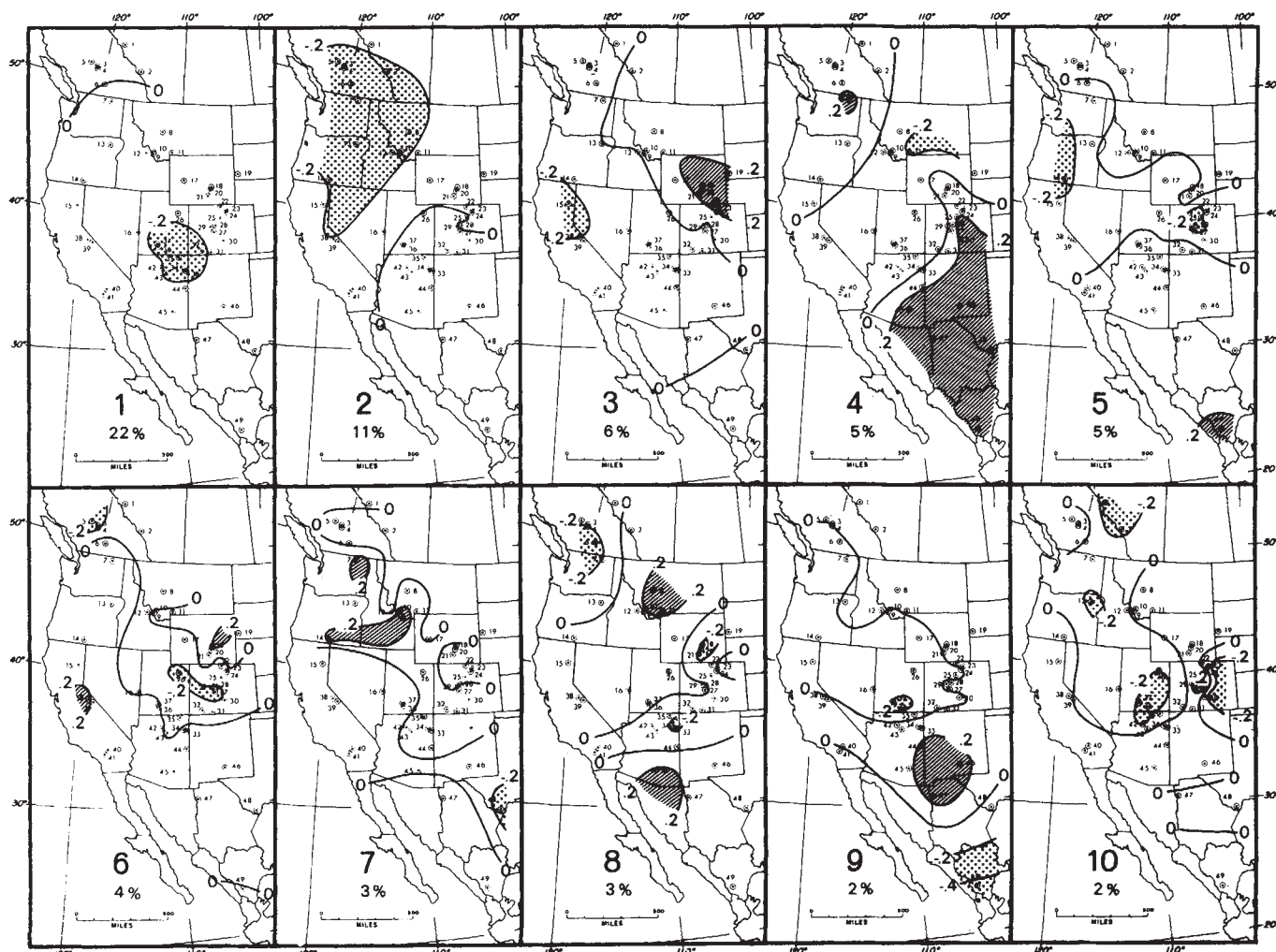
from 49 sites in western North America and albacore distribution both respond to the same variations in the ocean-atmosphere system, the large scale components of the ring-width variance were transformed into uncorrelated variables (amplitudes of principal component eigenvectors) and then put in stepwise regression to find which of these amplitudes were significant predictors (at the 95% confidence level) of albacore catch. This procedure gave the equation:

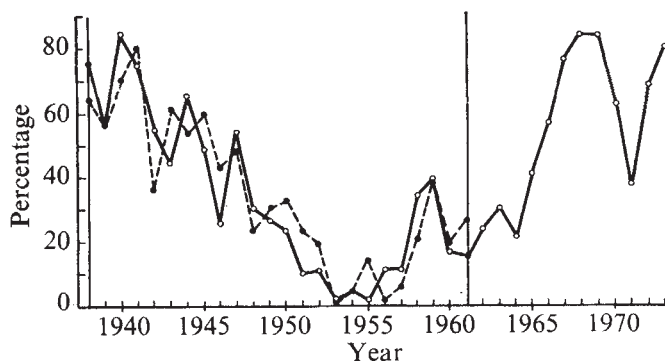
$$Y = 35.12 + 5.76A_2 + 5.13A_8 + 8.56A'_4 - 6.17A'_9 + 11.54A'_{10} \quad (1)$$

where  $Y$  is the estimated percentage of albacore caught north of San Francisco,  $A$  and  $A'$  are eigenvector amplitudes corresponding to the catch year and the following year, respectively, and subscripts refer to the eigenvector number (Fig. 1). The regression accounted for 83% of the catch data variance in the calibration period (Fig. 2). The values of tree-growth eigenvector amplitudes for 1700 to 1938 were then substituted into equation (1) to estimate what the yearly albacore catch distribution would have been for this early period based on the patterns of tree growth (Fig. 3).

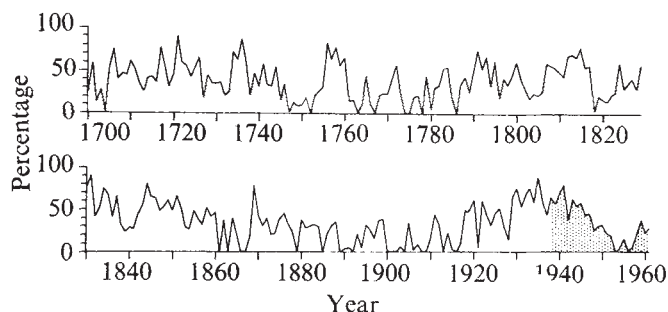
Although fishery statistics are available after 1961, tree-growth data for all 49 sites within the spatial network are not, and the only independent data available to check the validity of the reconstruction are incomplete fishery catch statistics between 1904 and 1937 (ref. 4). A qualitative comparison between both sets of data shows these common

Fig. 1 Maps of the first 10 eigenvectors of ring-width index. The percentage figures refer to the variance accounted for by the corresponding eigenvector. Numbered circles indicate ring-width chronology sites.





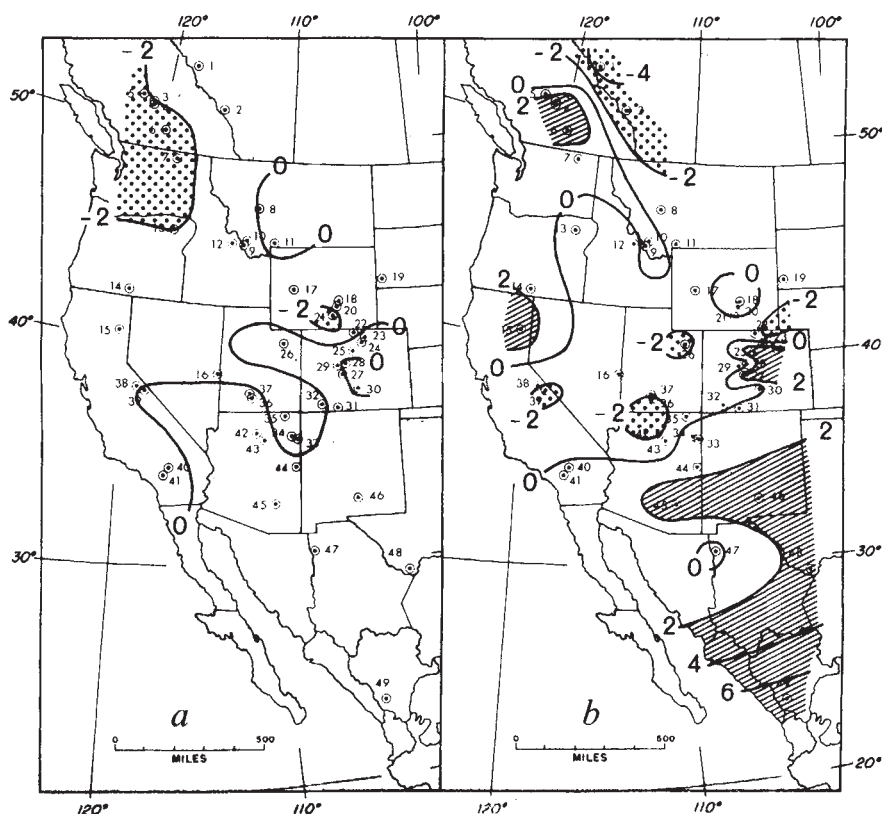
**Fig. 2** Percentage of the total North American west coast albacore tuna catch taken north of San Francisco, estimated from landings data (—) and percentage catch taken north of San Francisco derived from the calibration equation applied to tree-growth data over the dependent data period (---). Calibration included the following steps: (1) We computed the 49 by 49 correlation matrix which expressed the interrelationships among the 49 ring-width index series for the period of analysis (1700–1962). (2) The eigenvectors of the correlation matrix were then computed. These (orthogonal) eigenvectors may be expressed in map form as characteristic spatial anomaly patterns of tree growth. The 10 eigenvector patterns which accounted for the most variance (the large scale variance) were selected for further analysis. Maps of these eigenvectors are shown in Fig. 1. Linear combinations of these 10 eigenvectors can reproduce 64% of the ring-width variance for the 49 chronologies during the period of analysis. (3) Each of the first 10 eigenvectors was multiplied by the normalised tree-growth data at each site for each year from 1700 to 1962. The resulting matrix product consists of 10 time series of amplitudes which give the relative importance of each of the first 10 eigenvector patterns (the large scale patterns) in each year's tree-growth anomaly map. Being orthogonal to (uncorrelated with) each other they are easy to use as predictor variables in multiple regression. (4) Since trees respond to the climate of both the concurrent season and previous seasons, a prediction model was constructed including the 10 amplitudes corresponding to the same year as the catch data and the 10 amplitudes corresponding to the year following the catch data, for a total of 20 possible predictor variables. (5) Stepwise multiple regression was used to find those amplitudes that were significant predictors (95% confidence level) of albacore catch distribution for the years 1938–61.



**Fig. 3** Percentage of the total albacore tuna catch taken north of San Francisco, estimated from the calibration equation applied to tree-growth data for 1700 to 1961. Shading indicates calibration period.

characteristics: a fishery that was centred off southern California in the early 1900s; a sharp decrease in the availability of albacore to the southern fishery between 1917 and 1918; another decrease in availability of albacore to the southern fishery in 1923 when the total catch was the lowest since 1918; and a prolonged period of decreased availability to the southern fishing fleet after 1928. The reconstructed distribution data indicate that a fishery would have been centred quite far north after 1929 and through the late 1930s if boats had operated there during that period. The absence of albacore from the catch record for Oregon and Washington before 1937 and low catches for California after 1925 probably reflect the extreme northward and offshore population distribution indicated by the reconstructed data; the fish were just not available to salmon trollers that did operate close to shore north of California during this period<sup>3</sup>.

We also examined the eigenvector patterns of tree growth that were chosen by regression to be associated with a high percentage of albacore caught north of San Francisco. The tree-growth pattern corresponding to the year of the catch



**Fig. 4** Tree-growth anomaly patterns associated with above normal percentages of albacore caught north of San Francisco. Map *a*, corresponding to the year of the catch, is formed from the linear combination of eigenvectors 2 and 8 in equation (2); map *b*, corresponding to the year following the catch, is formed from the linear combination of eigenvectors 4, 9, and 10 in equation (3).



can be represented by a map composed of eigenvectors 2 and 8 as follows:

$$\text{map } a = 5.76 (\text{eigenvector } 2) + 5.13 (\text{eigenvector } 8) \quad (2)$$

where the numerical coefficients are taken from equation (1). Similarly, the tree-growth anomaly pattern corresponding to the year following a high percentage of albacore caught north of San Francisco can be represented as:

$$\text{map } b = 8.56 (\text{eigenvector } 4) - 6.17 (\text{eigenvector } 9) + 11.54 (\text{eigenvector } 10) \quad (3)$$

These maps are presented in Fig. 4. The ring-width data were mostly from trees sited in arid localities, so that a wide ring would generally be associated with anomalously cool, cloudy weather and above normal precipitation whereas a narrow ring would reflect warm, sunny and dry conditions.

Below normal tree growth in the Pacific North-west (Fig. 4) is indicative of dry conditions associated with below normal cyclonic activity during the fishing season. Sunny and mild weather would favour albacore fishing in adjacent waters, as would above normal insolation regardless of weather. The resulting excess of stored heat in the ocean would be given up through evaporation during the following autumn and winter and lead to increased cyclonic activity and precipitation along the coast north of San Francisco. These conditions would lead to increased tree growth during the following growing season (Fig. 4).

Autumn and winter climatic anomaly features, combined with spring climate and the year-to-year autocorrelation of tree-ring widths, produce the other ring-width anomaly features in Fig. 4 for the following growing season. Narrow ring widths south of San Francisco, for example, imply below normal precipitation—an expected feature since winter precipitation in the Pacific North-west is negatively correlated with winter precipitation in southern California<sup>9</sup>.

The reconstructed values of albacore catch distribution data (Fig. 3) and inferred population distribution also seem to exhibit long term changes over intervals of 100 yr or more, which suggest the possibility that long term fluctuations in the ocean-atmosphere system may be involved.

The success of the calibration of tree rings with albacore catch indicates the possibility of relating tree-ring variations to any type of biological variations which are affected by large scale climatic fluctuations. Such relationships may be quantified and used to reconstruct objectively other climatically-caused biotic variations in the past.

N. E. CLARK

National Oceanic and Atmospheric Administration,  
National Marine Fisheries Service,  
Southwest Fisheries Center,  
La Jolla, California 92037

T. J. BLASING  
H. C. FRITTS

Laboratory of Tree-Ring Research,  
University of Arizona, Tucson, Arizona 85721

Received December 5, 1974; accepted May 6, 1975.

- <sup>1</sup> LaMarche, V. C., *Science*, **183**, 1043–1048 (1974).
- <sup>2</sup> Namias, J., *Mon. Weath. Rev.*, U.S. Dep. Agric., **97**, 173–192 (1969).
- <sup>3</sup> Laurs, R. M., et al., *Report of Joint National Marine Fisheries Service–American Fishermen's Research Foundation Albacore Studies Conducted During 1973* (National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, 1973).
- <sup>4</sup> Clemens, H. B., and Craig, W. L., *Calif. Dept Fish and Game, Fish Bull.*, **128** (1965).
- <sup>5</sup> Sette, O. E., *Calif. Coop. Oceanic Fish. Invest. Rept.*, **7**, 181–194 (1960).
- <sup>6</sup> Pyke, C. B., *An Investigation of some precipitation patterns in California and adjacent regions* (University of California Water Resources Center, 1966).

## Oats may grow better in water depleted in oxygen 18 and deuterium

WHILE growing oats at different temperatures in water of different <sup>18</sup>O and deuterium (D) abundances, we noticed that oats grown in Antarctic water in which is depleted in <sup>18</sup>O and D by –49‰ and –400‰, relative to standard mean ocean water (SMOW used as a comparative reference in hydrogen and oxygen isotope studies), showed initial growth 1–2 weeks sooner than did oats grown in water containing greater <sup>18</sup>O and D concentrations. The oats seemed to grow better in water which was most depleted in the stable isotopes throughout the growth period.

The oats were grown from the same batch of seeds in two sealed glass-covered glass jars (approximately 10 l). Twenty-five oat seeds were added to each jar, containing the same amount of vermiculite and 500 ml water to which 5.0 g Rapid-Gro, a commercial fertiliser, had been added. One jar contained melted glacial ice from the Antarctic with isotope concentrations of –49‰ δ<sup>18</sup>O (SMOW) and –400‰ δD (SMOW). The other jar contained distilled ocean water with +1.0‰ δ<sup>18</sup>O (SMOW) and +17‰ δD (SMOW). Both jars were placed in the chamber at the same time.

The experiment was repeated three times with new materials: once the growth chamber was maintained between 1.7 and 3.3 °C; once between 24 and 26.6 °C; and once the temperature fluctuated between 1.7 and 26.6 °C. Each time the oats in the jar containing water depleted in the heavy isotopes showed germination 1–2 weeks earlier and seemed to grow better throughout the growth period, than oats grown in distilled ocean water.

Using oats grown at 15 °C, the first sign of germination in the jar containing water depleted in the heavy isotopes was 4 d after planting. On the day 6, eight plants (out of 25) had attained a height of 6 cm. The first sign of germination in the jar with water containing the heavier isotope concentration, was after 17 d. By the time five plants had attained a height of 6 cm in this jar, in that with water depleted in the isotopes, 23 plants that had reached the top of the jar (approximately 25 cm).

Kashutin<sup>1</sup> observed that snow-water depleted in D increases the yield of cucumbers, radishes and spring wheat compared with controls grown in ordinary water of unspecified isotopic composition. He cites experiments on the egg productivity of hens and the weight gain of suckling pigs. In both cases water depleted in D was especially efficient in promoting productivity.

Although much has been done on the effect of D-enriched water on biological systems, we suggest that research on the effect D-depleted water on plant and animal growth may prove fruitful. A major source of water depleted in D by over 400‰ (40%) compared with SMOW is snow and ice from the Antarctic polar plateau. Water depleted by 150–180‰ is readily available in the USA from Rocky Mountain snow precipitating above 10,000 feet elevation.

JIM D. GLEASON  
IRVING FRIEDMAN

US Geological Survey,  
Denver, Colorado 80225

Received February 10; accepted June 3, 1975.

<sup>1</sup> Kashutin, K., *Priroda (USSR)*, **58**, 107 (1969).

## Identification of chlorinated dibenzofurans in American polychlorinated biphenyls

MORTALITY of embryos has contributed to the reproductive failures of several bird species, including the sparrowhawks (*Accipiter nisus*) of southern Scotland<sup>1</sup>, the white-tailed eagles (*Haliaeetus albicilla*) of Schleswig Holstein<sup>2</sup>, and the herring

gulls (*Larus argentatus*) of Lake Ontario<sup>3</sup>. Suspected causes include *p,p'*-DDE (2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene), other chlorinated biocides and/or their derivatives, and the polychlorinated biphenyls (PCBs), all of which are present as contaminants in the eggs<sup>1-3</sup>. PCBs are present in high concentrations in the bird populations which suffer embryonic mortality<sup>1-3</sup>. Other organochlorine compounds which may be present in food webs include the chlorinated dibenzodioxins and the chlorinated dibenzofurans (Fig. 1), which are toxic to embryos in amounts<sup>4-7</sup> less than 1 µg. They are therefore among the most toxic substances known and are possible causes of the observed mortality.

The chlorinated dibenzodioxins and chlorinated dibenzofurans, however, have proved exceedingly difficult to detect in environmental samples in the concentrations at which they are expected to be embryotoxic<sup>8-10</sup>. The chlorinated dibenzodioxins enter the environment as contaminants in preparations of the herbicide 2,4,5-T (refs 5 and 11) and the fungicide pentachlorophenol<sup>12,13</sup>. Chlorinated dibenzofurans have been found in a French (Phenoclor DP6) and a German (Clophen A60) PCB and were shown to be the active embryotoxic agent in these preparations<sup>6</sup>. The techniques used, however, did not detect chlorinated dibenzofurans in an American PCB, Aroclor 1260. We report here the presence of chlorinated dibenzofurans in Aroclor PCB, widely used in North America and Great Britain, and in the same Aroclor 1260 preparation examined previously with negative findings<sup>6</sup>.

Samples of PCB examined include: Aroclor 1248, 1254, and 1260 (1969); Aroclor 1254 (1970); Aroclor 1016 (1972); and the same three preparations studied by Vos *et al.*<sup>6</sup>: Aroclor 1260, lot No. AK-3; Clophen A-60, lot No. 912434; and Phenoclor DP-6, lot not specified. The latter three PCBs were obtained from Dr Vos, the others from the Monsanto Company in the years indicated in parentheses.

PCBs extracted from environmental samples most often have gas chromatographic profiles similar to those of PCB formulations containing approximately 48, 54 or 60% chlorine. In the Aroclor series, the former two PCBs are equivalent to Aroclor 1248 and Aroclor 1254, respectively. Aroclor 1260, Phenoclor DP6, and Clophen A60 all contain approximately 60% chlorine.

Chlorinated dibenzofurans were identified in all Aroclor preparations except Aroclor 1016, as well as in Clophen A60 and Phenoclor DP6. Aroclor 1016 is a PCB mixture containing

**Table 1** Chlorinated dibenzofuran concentrations\* in Aroclor, Clophen and Phenoclor

|                         | PCB | 4-Cl     | 5-Cl      | 6-Cl     | Total |
|-------------------------|-----|----------|-----------|----------|-------|
| Aroclor 1248 (1969)     |     | 0.5 (25) | 1.2 (60)  | 0.3 (15) | 2.0   |
| Aroclor 1254 (1969)     |     | 0.1 (6)  | 0.2 (12)  | 1.4 (82) | 1.7   |
| Aroclor 1254 (1970)     |     | 0.2 (13) | 0.4 (27)  | 0.9 (60) | 1.5   |
| Aroclor 1260 (1969)     |     | 0.1 (10) | 0.4 (40)  | 0.5 (50) | 1.0   |
| Aroclor 1260 (lot. AK3) |     | 0.2 (25) | 0.3 (38)  | 0.3 (38) | 0.8   |
| Aroclor 1016 (1972)     | ND  | ND       | ND        | ND       | —     |
| Clophen A-60            |     | 1.4 (17) | 5.0 (59)  | 2.2 (26) | 8.4   |
| Phenoclor DP-6          |     | 0.7 (5)  | 10.0 (74) | 2.9 (21) | 13.6  |

\*Expressed as µg g<sup>-1</sup> PCB. Values in parentheses represent quantity as percentage total dibenzofuran.

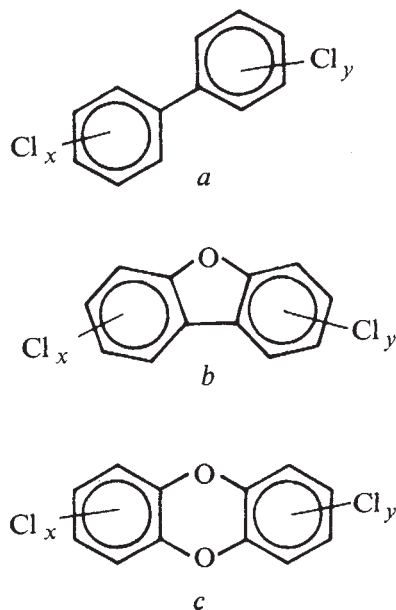
ND, not detected (<0.001 µg g<sup>-1</sup>).

Amounts of PCB ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (180 g, internal diameter 31.5 mm), and eluted with: an additional 1,600 ml hexane, and successive 800 ml volumes each of 5% diethyl-ether-hexane, 25% diethyl-ether-hexane and acetone, at a rate of approximately 7 ml min<sup>-1</sup>. The major portion of the PCB was eluted in the hexane fraction, which was discarded. On addition of the 5% mixture, the eluates were collected in six successive 400 ml volumes. To eliminate the polar solvents, each eluate was evaporated twice just to dryness and taken up each time in a minimal amount of hexane. Each fraction, in 1 ml hexane, was placed on a microalumina column<sup>14</sup> and eluted with 10 ml each of 1% and 20% methylene chloride in hexane. These were also taken twice just to dryness and made to up a volume of 1 ml in hexane to eliminate the methylene chloride before gas chromatographic analysis. Aliquots of all fractions obtained before and after partitioning on alumina were injected into a six foot glass column containing 3% OV1 on 100–120 mesh Supelcoport in Tracor MT220 and Hewlett-Packard 5700 gas chromatographs equipped with <sup>63</sup>Ni electron-capture detectors. PCBs were found to be present in each fraction eluted from the Florisil column in amounts sufficient to interfere with the detection of trace contaminants. Partitioning on the alumina columns separated most of the PCB interference into the 1% methylene chloride fractions. On removal of this interference, different peak patterns appeared in the chromatograms of the 20% methylene chloride fractions. Compounds eluting in the 20% methylene chloride fraction were collected for mass spectrometric analysis using a 20:1 effluent splitter, and a trap consisting of a capillary tube (1 mm internal diameter, 100 mm long) bent to a U shape, immersed in a liquid nitrogen bath. Methylene chloride (20%; 4 µl) in hexane was injected into the capillary as a rinse, removed with a 1.0 µl micropipette, and placed directly on the mass spectrometer probe. The probe was inserted into a GEC AEI MS902 high resolution mass spectrometer and the solvent removed by the force pump. The probe was rapidly inserted into the ion source and multiple scans were recorded in the on-line high resolution mode<sup>15</sup>.

approximately 42% chlorine and has replaced Aroclor 1242 in many applications, principally as the dielectric fluid in capacitors<sup>16</sup>. Values reported in Table 1 represent the total of those compounds found in 400 ml Florisil fractions 2–6. A total of 10–12 isomers was identified in each PCB. Two chlorinated dibenzofuran contaminants have been reported for the Clophen and Phenoclor previously<sup>6</sup>; our first analyses of the Clophen revealed an additional five chlorinated dibenzofurans<sup>8</sup>. The structures contained four to six chlorine atoms. Other dibenzofurans including those chlorinated to a lesser extent may have been present in the first 400 ml fraction but this was not examined in detail as it contained substantial PCB interference. Recently synthesised 2,3,7,8-tetra-, 2,3,4,7,8-penta- and 2,3,4,6,7,8-hexachlorodibenzofuran were used to quantify tetra-, penta-, and hexachlorodibenzofurans, respectively. The former two authentic standards had retention times on the OV1 column the same as those of two dibenzofurans isolated from the PCB.

Vos *et al.*<sup>6</sup> detected no chlorinated dibenzofurans in an Aroclor 1260 preparation at a detection limit of 1 p.p.m. Fractionation and examination of the identical Aroclor 1260 in our study confirm their findings based on the stated limit, but reveal the presence of 11 chlorinated dibenzofurans in the preparation, having a total concentration of 0.8 µg g<sup>-1</sup> PCB (Table 1). The same workers also found diethyl ether extracts of the Clophen A60 and Phenoclor DP6 to be much more toxic to chick embryos than diethyl ether extracts of Aroclor 1260. Our study confirms those findings on the basis of chlorinated

**Fig. 1** Skeletal structures of: *a*, chlorinated biphenyl,  $x+y = 1-10$ ; *b*, chlorinated dibenzofurans,  $x+y = 1-8$ ; *c*, chlorinated dibenzodioxins,  $x+y = 1-8$ .





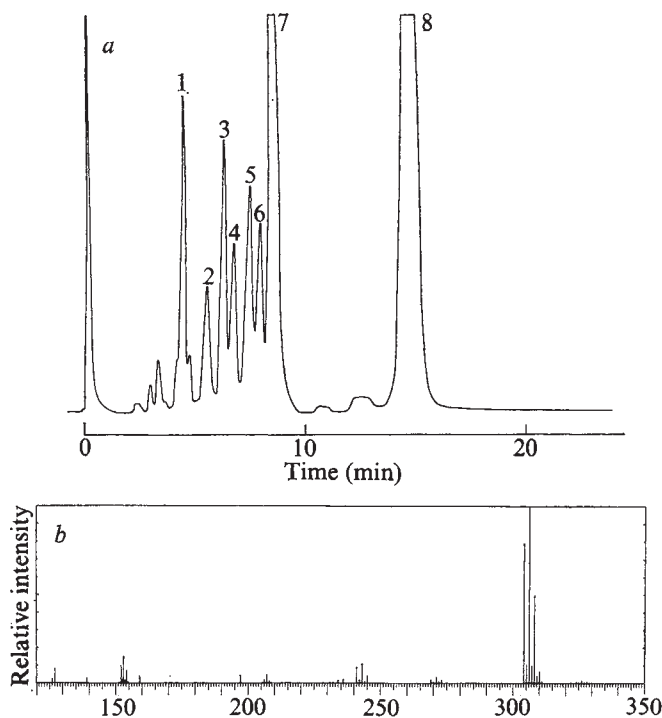


Fig. 2 a, Gas chromatogram of a fraction of Aroclor 1254 containing a mixture of chlorinated biphenyls, dibenzofurans, and naphthalenes. Identities of peaks are given in the text. b, Mass spectrum of peak 2, a tetrachlorodibenzofuran.

dibenzofuran content: the identical Clophen and Phenoclor contain 11 and 17 times more total chlorinated dibenzofurans, respectively, than the Aroclor 1260.

A gas chromatogram showing components derived from the Aroclor 1254 obtained in 1969 is represented in Fig. 2. The components were eluted in the second 400 ml Florisil fraction and recovered from the alumina column in 20% methylene chloride-hexane. Each of the numbered peaks was trapped as described here, and identified by mass spectrometric analysis. A nominal mass plot of the high resolution mass spectrum of peak 2 is shown in Fig. 2. The plot includes all the ions with elemental compositions ranging to the maximum empirical formula  $C_{13}H_6O^{36}Cl_5^{37}Cl_4^{13}C_2$ . The molecular ion cluster at nominal  $m/e$  304–310 fragments by successive losses of Cl to yield the ions at  $m/e$  269–275 and CO to the ions at  $m/e$  241–245. A minor loss of Cl from the peaks at  $m/e$  269–275 also occurs to yield the ions at  $m/e$  234–238, followed by CO elimination to  $m/e$  206–210.

The group of peaks at  $m/e$  152–154 are the doubly charged molecular ions. An identical spectrum was obtained from an authentic standard of 2,3,7,8-tetrachlorodibenzofuran. This latter compound has a retention time identical to that of peak 4. Peak 2 is, therefore, a positional isomer. The accurate mass measurements for the characteristic ions are within 2 p.p.m. of the calculated exact masses. Peaks identified on this chromatogram and their retention times relative to dieldrin are as follows: a mixture of tetra- and pentachlorobiphenyl (1.02); tetrachlorodibenzofuran (1.30); pentachlorobiphenyl (1.46); tetrachlorodibenzofuran (1.57); hexachloronaphthalene (1.75); pentachlorobiphenyl (1.86); hexachloronaphthalene (2.00); and heptachloronaphthalene (3.46). An aliquot of combined fractions derived from the same Aroclor 1254 was treated with diazomethane to assess whether any chlorinated ortho-hydroxybiphenyls (pre-furans) were present. Gas chromatographic analysis of the sample before and after treatment resulted in identical chromatograms.

As large quantities of PCBs have entered the global environment<sup>17–20</sup>, it may be assumed that the contaminant dibenzofurans

also have been released in proportional amounts. Their persistence, effects, and significance remain to be determined.

We thank J. A. Burke, M. L. Porter and J. G. Vos for discussions; A. S. Kende for standards of chlorinated dibenzofurans; and F. C. Walls for assistance with the mass spectrometry. This work was supported by the Canadian Wildlife Service, National Science Foundation, and NASA.

GERALD W. BOWES\*

MICHAEL J. MULVIHILL

Canadian Wildlife Service,  
Toxic Chemicals Section,  
Ottawa, Canada K1A 0H3

BERND R. T. SIMONEIT

A. L. BURLINGAME

Space Sciences Laboratory,  
University of California,  
Berkeley, California 94720

R. W. RISEBROUGH

Bodega Marine Laboratory,  
University of California,  
Bodega Bay, California 94923

Received February 13; accepted May 28, 1975.

\*Present address: California Water Resources Control Board, Division of Planning and Research, 1416 Ninth Street, Sacramento, California 95814.

- 1 Newton, I., and Bogan, J., *Nature*, **249**, 582–583 (1974).
- 2 Koeman, J. H., Hadderingh, R. H., and Bijleveld, M. F. J., *Biol. Conserv.*, **4**, 373–377 (1972).
- 3 Gilbertson, M., and Hale, R., *Can. J. Zool.*, **88**, 354–356 (1974).
- 4 Higginbotham, G. R., et al., *Nature*, **220**, 702–703 (1968).
- 5 Sparschu, G. L., Dunn, F. L., and Rowe, V. K., *Food Cosmet. Toxicol.*, **9**, 405–412 (1971).
- 6 Vos, J. G., Koeman, J. H., Van der Maas, H. L., ten Noever de Brauw, M. C., and de Vos, R. H., *Food Cosmet. Toxicol.*, **8**, 625–633 (1970).
- 7 Vos, J. G., *Environ. Hlth Persp.*, **1**, 105–117 (1972).
- 8 Bowes, G. W., Simoneit, B. R., Burlingame, A. L., de Lappe, B. W., and Risebrough, R. W., *Environ. Hlth Persp.*, **5**, 191–198 (1973).
- 9 Baughman, R., and Meselson, M., *Environ. Hlth Persp.*, **5**, 27–35 (1973).
- 10 Baughman, R., and Meselson, M., *Adv. Chem.*, **120**, 92–104 (1973).
- 11 Report on 2,4,5-T (Executive Office of the President, Science Advisory Committee, Office of Science and Technology, March, 1971).
- 12 Jensen, S., and Renberg, L., *Ambio*, **1**, 62–65 (1972).
- 13 Firestone, D., Riss, J., Brown, N. L., Barron, R. P., and Damico, J. N., *J. Ass. Off. Anal. Chem.*, **55**, 85–92 (1972).
- 14 Porter, M. L., and Burke, J. A., *J. Ass. Off. Anal. Chem.*, **54**, 1426–1428 (1971).
- 15 Burlingame, A. L., Olsen, R. W., and McPherron, R. V., *Adv. Mass Spectr.*, **6**, 1053–1059 (1974).
- 16 Nisbet, I. C. T., and Sarofim, A. F., *Environ. Hlth Persp.*, **1**, 21–38 (1972).
- 17 Bowes, G. W., and Jonkel, C. J., *J. Fish. Res. Bd Can.* (in the press).
- 18 Jensen, S., Johnels, A. G., Olsson, M., and Otterlind, G., *Nature*, **224**, 247–250 (1969).
- 19 Koeman, J. H., ten Noever de Brauw, M. C., and de Vos, R. H., *Nature*, **221**, 1126–1128 (1969).
- 20 Risebrough, R. W., Reiche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., *Nature*, **220**, 1098–1102 (1968).

## Niche breadth in Bryozoa as a test of competition theory

COMPETITION theory predicts that intraspecific and interspecific competition should often have opposite effects on the use of resources by a population, the former increasing, the latter decreasing, the range of resource actually used<sup>1,2</sup>. Field data supporting these predictions are well known for the interspecific case<sup>3,4</sup> but are scarce for the intraspecific condition, and we have been unable to find any reference demonstrating both effects within a single species. We therefore report here the verification of both predictions in respect of competition for space by the epiphytic bryozoan *Alcyonidium hirsutum*; less extensive data suggesting the same effects within other bryozoans are also reported.

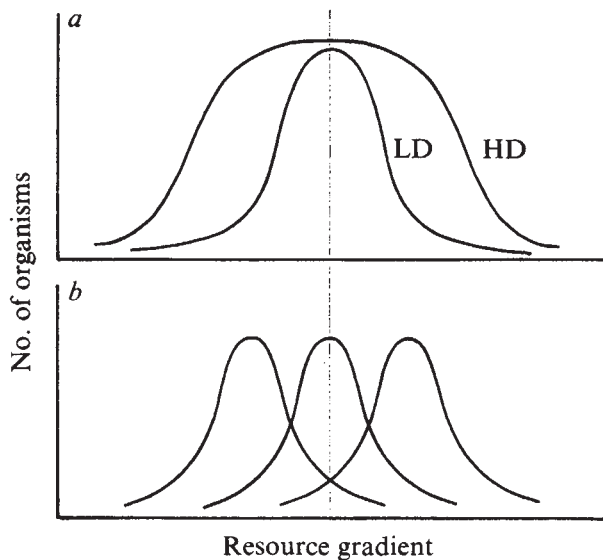
Intraspecific competition should result in an increase in the range of a resource spectrum used by a species, as at high population levels the advantages to any individual of being at the competition-free optimum of a resource gradient are offset by the intense intraspecific competition found there (Fig. 1a); this is the 'principle of equal opportunity' of MacArthur<sup>1</sup>. Interspecific competition, on the other hand, should tend to restrict the range of the resource spectrum used by a species, as individuals attempting to exploit marginal resources cannot do so as efficiently as



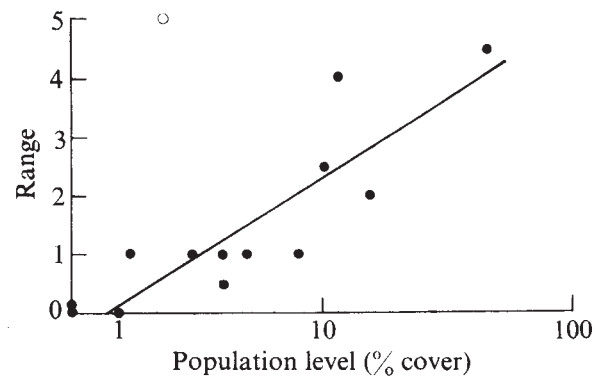
individuals of other species for which these resources are more nearly optimal; niche width on the resource gradient is thus reduced. Furthermore, such reduction can be expected to increase with increase in the number of competing species, as a greater proportion of the original resources is then nearer the optimum for a competing species (Fig. 1b); this is the diffuse competition situation of MacArthur<sup>1,2</sup>. These predictions will have been confirmed if it can be shown that the range of a resource spectrum used by a species increases with population density but decreases as the number of competing species increases.

Our analysis is based on the distributions of Bryozoa on plants of the serrated wrack *Fucus serratus*. Sixty-five plants were collected in April, 1972 near Ardkeen, County Down, Northern Ireland, and the longest frond of each divided into Y-shaped dichotomies, labelled Y1, Y2, and so on, from holdfast to tip. The percentage cover by each bryozoan species was recorded for each of the two faces of each dichotomy. The commonest bryozoans were *Alcyonidium hirsutum*, *Electra pilosa*, *Flustrellidra hispida* and *Membranipora membranacea*; other bryozoan species were sufficiently rare to be ignored in this analysis. Fuller details of our methods have been recorded elsewhere<sup>3</sup>.

**Fig. 1** Niche breadth changes under the influence of intraspecific (a) and interspecific (b) competition. In a, high density (HD) curve is broader than is the low density (LD) curve as more individuals settle at more marginal but less hotly contested points on the resource gradient. In b, presence of competitors utilising resources on both sides of a species' optimum on the resource gradient compresses that species' niche breadth.



In an epiphytic community of filter-feeders such as that found on *F. serratus*, competition for space is of major significance: each fucoid frond constitutes a linear resource which can be partitioned longitudinally among the competing species<sup>3,6</sup>. We therefore tested MacArthur's principle of equal opportunity by plotting the longitudinal range of *Alcyonidium* on each plant against its population density on that plant, using only the fourteen plants on which it was the sole bryozoan present and thus eliminating the effects of interspecific competition from other bryozoans. The results (Fig. 2) show that *Alcyonidium* used a greater range of Y values (height when the plants are upright, as an index of distance from the holdfast) at high population densities than at low densities. As the available space at the optimum height was not physically filled as populations increased this must result from competitive interactions between colonies<sup>7</sup>, thus confirming the prediction of increased range of resource use. Note that



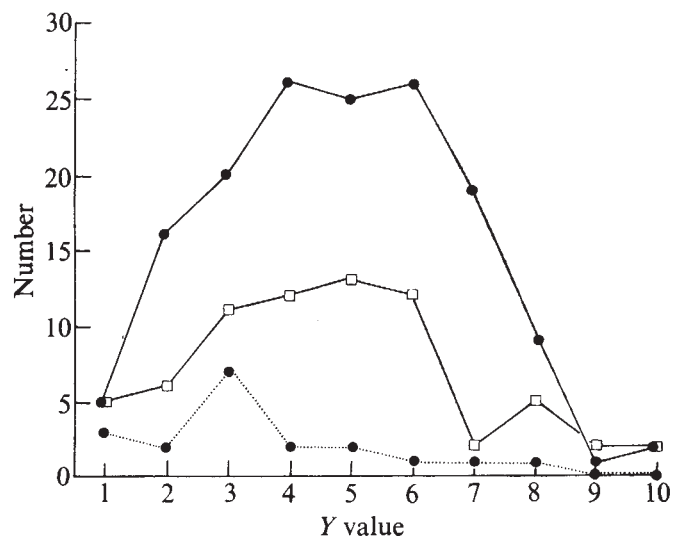
**Fig. 2** Niche breadth in relation to population level for *Alcyonidium* in the absence of competition from other bryozoa. Niche breadth estimated as the range in Y value of colonised segments, calculated separately for each side of a frond and averaged. Population level is estimated as the mean percentage cover of all colonised segment faces of the plant. ○, Anomalous result discussed in text. Regression is significant ( $r = 0.631$ ,  $P < 0.05$ ).

one plant gave anomalous results because of extremely distal settlement of a very few zooids (Fig. 2); the regression is statistically significant when this point is included ( $r = 0.631$ ,  $P < 0.05$ ) but on omitting it the correlation rises to 0.890 ( $P < 0.01$ ), thus almost doubling the proportion of variance in resource span accounted for by population density.

Too few plants were colonised by another single bryozoan species to allow precisely the same analysis as for *Alcyonidium*. For these other species we therefore included plants on which any one other bryozoan was present even though this introduced some interspecific competition tending to reduce niche breadths (see below) and thus to mask the intraspecific effect. Nevertheless, for each species a positive correlation between range and density per plant (on a log scale) was obtained (*Electra*,  $r = 0.595$ ,  $P < 0.025$ ; *Flustrellidra*,  $r = 0.363$ ,  $P < 0.30$ ; *Membranipora*,  $r = 0.606$ ,  $P < 0.09$ ), thus suggesting a niche widening effect for intraspecific competition in these species also.

It is in principle possible for the results above to have arisen through a pattern of age-dependent settlement by Bryozoa on growing *Fucus* fronds<sup>3</sup>. We therefore examined

**Fig. 3** Resource utilisation curves for *Alcyonidium* with different numbers of competing Bryozoa present. Ordinate, number of *Fucus* segment faces colonised; abscissa, Y value. ●—●, Faces colonised by *Alcyonidium* alone; □—□, faces colonised by *Alcyonidium* and any one other bryozoan species; ●...●, faces colonised by *Alcyonidium* and two other bryozoans.



**Table 1** Effect of interspecific competition on niche breadth in Bryozoa

| Number of competing species present | <i>Alcyonidium</i> |       | <i>Electra</i> |       | <i>Flustrellidra</i> |       | <i>Membranipora</i> |       |
|-------------------------------------|--------------------|-------|----------------|-------|----------------------|-------|---------------------|-------|
|                                     | Breadth            | Cases | Breadth        | Cases | Breadth              | Cases | Breadth             | Cases |
| 0                                   | 5.9                | 149   | 5.1            | 59    | 6.8                  | 51    | 5.0                 | 41    |
| 1                                   | 4.4                | 69    | 2.6            | 58    | 6.1                  | 57    | 1.7                 | 18    |
| 2                                   | 2.7                | 18    | 1.6            | 20    | 1.6                  | 20    | 1.0                 | 2     |

Niche breadth was assessed from plots such as those of Fig. 3 as the width of the resource utilisation function at half-height. Cases column shows the sample size on which each function was based. The second and third rows are based on all cases satisfying the row criterion, irrespective of the identity of the competitor(s).

the distribution of each species on each plant in the light of the age of that plant as indexed by the number of dichotomies on the longest frond: no systematic pattern was apparent in any case, thus precluding that explanation.

The effects of interspecific competition on *Alcyonidium* niche breadth were examined by plotting the distribution of segment faces colonised by *Alcyonidium* with (in turn) no other, one other and two other bryozoan species on the same face. The results (Fig. 3) show that *Alcyonidium* used a wider range of heights on the plants in the absence of competition than in its presence, and used a wider range when competing with one species at a time than with two. These results are summarised with those for the other three species in Table 1, using the width at half-height of each resource utilisation function as an index of niche breadth; the standard deviations used for this purpose by MacArthur<sup>1</sup> were not appropriate with the non-Gaussian distribution found here<sup>8</sup>. Table 1 shows that niche breadth was reduced progressively in all four species as the number of competing species increased, thus confirming the theoretical prediction for the effects of interspecific competition. Note that these results did not arise as artefacts of partial overlapping of largely segregated resource utilisation functions<sup>9</sup>.

A number of previous studies, particularly those demonstrating ecological release in island populations of birds<sup>3,4</sup>, have shown interspecific competition to restrict niche breadth. The effect of intraspecific competition in increasing niche breadth has been less well documented, although it can be inferred from the 'buffer effect' of population studies, in which marginal habitats are utilised by territorial animals only at high population levels<sup>9</sup>. These two types of study have rarely been combined to contrast the effects of interspecific and intraspecific competition within a single species, probably because of the practical difficulties of assessing population density and resource use simultaneously. The more rigorous test of competition theory reported here was possible because of the special features of the group studied, bryozoans on *Fucus* competing along what is effectively a single resource dimension on which their densities are readily assessed. A detailed account of habitat partitioning in bryozoans will be published elsewhere.

R. J. O'CONNOR  
P. J. S. BOADEN  
R. SEED

Department of Zoology and Marine Biology Station,  
The Queen's University of Belfast,  
Belfast BT7 1NN, Northern Ireland

Received April 16; accepted May 28, 1975.

<sup>1</sup> MacArthur, R. H., *Geographical Ecology* (Harper and Row, New York, 1972).

<sup>2</sup> Pianka, E. R., *Evolutionary Ecology* (Harper and Row, New York, 1974).

<sup>3</sup> Crowell, K. L., *Ecology*, 43, 75–88 (1962).

<sup>4</sup> Lack, D., *Ibis*, 86, 260–286 (1944).

<sup>5</sup> Boaden, P. J. S., O'Connor, R. J., and Seed, R., *J. exp. mar. Biol. Ecol.*, 17, 111–136 (1975).

<sup>6</sup> Boaden, P. J. S., O'Connor, R. J., and Seed, R., *J. exp. mar. Biol. Ecol.* (in the press).

<sup>7</sup> Ryland, J. S., *Bryozoans* (Hutchinson, London, 1970).

<sup>8</sup> Cody, M. L., *Competition and the Structure of Bird Communities* (Princeton University Press, Princeton, 1974).

<sup>9</sup> Krebs, J. R., *Ecology*, 52, 2–22 (1971).

## Hue is an absolute code for young children

THE question of how young children see, remember, and learn represents a recurring theme in psychology. Bryant<sup>1</sup> has argued provocatively that "young children can on the whole register and remember relative values with great ease, but have problems in situations in which they must remember absolute values along any continuum". Bryant shows<sup>1</sup> how this view of early cognitive functioning accounts for the results from studies of children's responses to several environmental continua of a perceptual nature (for example, orientation, size, and position). I have, however, observed that young infants respond to changes of wavelength in a manner which indicates that they code hue absolutely.

Bryant has reanalysed extant studies and described new data which show that children perform well at simultaneous discrimination tasks (where comparison stimuli are available at the same time as a standard) whereas they perform poorly at successive discrimination tasks (where choice stimuli are available after a standard has been withdrawn). On the basis of this evidence, Bryant proposed that children use relative codes to make simultaneous comparisons. Successful performance in successive comparisons requires a constant external framework or an absolute code of stimulus characteristics; in general, Bryant has found that where mediating environmental features were wanting children's performances at successive discriminations fell to chance. Relative codes rather than absolute codes, he concluded, are therefore primary in young children, and the growing reliance on absolute codes, if it occurs at all, is a late developmental phenomenon. In this view, man's initial perception and memory of environmental stimuli are dominated and controlled exclusively by their relationships to each other or to surrounding frames of reference.

Contrary to Bryant's analysis, for a stimulus dimension (wavelength) which conforms to his investigative requirements (both absolute values of stimuli and relationships among stimuli can be specified) and in an experimental situation (successive discrimination) which fits his physical requirements (luminous figures were presented without a frame of reference in an otherwise dark room), infants in my studies were observed to use absolute codes in successive discrimination. That is, infants responded to hues on an absolute basis with no alternatives other than those which may be inferred to be internal categories of information. This is much the way that adults respond to hue<sup>2–3</sup>.

Ten infants (mean age, 124 d) were first familiarised with a 480 nm stimulus: they were shown a light—which appears to the adult mostly 'blue' with some 'green'—serially for 15 trials each of 15 s duration. During this



familiarisation phase (and during the test phase which followed) the infants' observation of the light was monitored by observers unaware of the stimulus wavelength (judgment reliabilities:  $0.93 < r < 0.97$ ), and the total observation times per trial were later scored blindly from event records. Typically, familiarisation to one stimulus leads to shorter periods of observation on successive presentations of that stimulus; after familiarisation, continued exposure to the original stimulus or to a different stimulus coded as the same as the first leads to a continued depressed level of looking, whereas exposure to a different stimulus coded differently from the first leads to increased visual attention<sup>4</sup>. Immediately following the familiarisation phase, infants experienced nine test trials (each of 15 s duration) in which they saw 450 nm, 480 nm, and 510 nm stimuli serially in three different random orders. Thus, in the test phase, infants saw the familiarisation stimulus (480 nm or 'greenish-blue') and two new stimuli: 450 nm or 'blue' and 510 nm or 'green'. Adults see and code 450 nm and 480 nm as similar and as drawn from the same basic hue category, but they see and code 480 nm and 510 nm as different and as drawn from different basic hue categories. This experiment (Table 1) showed that infants looked at 450 nm and 480 nm for an equal and relatively little amount of time but that they looked at 510 nm for significantly longer ( $F=16.77$ ,  $P<0.001$ , by a planned comparisons analysis<sup>5</sup>).

In a successive discrimination task in a situation lacking external frames of reference, infants responded to equal extents to a wavelength with which they had been previously familiarised and to another from the same basic hue category, which they had not seen previously; but they responded to a greater extent than that to a wavelength which they had not seen previously and which was drawn from a different hue category. Internal controls showed that differential responses could not be a function of fluctuation in attention (the familiarisation stimulus appeared randomly during the test) or simple change in wavelength (the two new stimuli were equally distant in wavelength from the familiarisation stimulus). Moreover, in a similar successive discrimination task, infants who were familiarised with a luminous oblique line did not discriminate it from its mirror image; that is, they gave equal amounts of attention to both. It is clear from this pattern of results that infants can code wavelength absolutely by apparent hue and can respond to changes in wavelength with respect to a set of basic absolute hue codes, whereas they cannot code orientation absolutely<sup>1</sup>.

It is an explicit claim of Bryant's theory that young children, possessing exclusively relative codes, should be able to perform successive discrimination tasks and concomitant perceptual inferences only in the presence of a constant frame of reference. Without such a reference, absolute codes are necessary, and lacking such codes children perform at chance. The possession by infants of an absolute code for hue (and for certain speech sounds<sup>6</sup>) represents, however, a case which Bryant happily provided for: "Abilities which have been definitively ruled out in young children by one psychologist have a habit of cropping up in the experiments of another". The presence of hue codes in young infants helps us further understand the

nature of early sensory and perceptual processes and of the demonstrative predominance of colour over form in young children's natural classification systems<sup>7</sup>.

MARC H. BORNSTEIN

Department of Psychology,  
Yale University,  
New Haven, Connecticut 06520

Received April 4; accepted May 28, 1975.

<sup>1</sup> Bryant, P. E., *Perception and Understanding in Young Children* (Basic Books, New York, 1974).

<sup>2</sup> Bornstein, M. H., *Psychol. Bull.*, **80**, 257 (1973).

<sup>3</sup> Boynton, R. M., *Woodworth and Schlosberg's Experimental Psychology* (edit. by

Kling, J., and Riggs, L. A.), 315 (Holt, Rinehart, and Winston, New York, 1973).

<sup>4</sup> Jeffrey, W. E., and Cohen, L. B., *Adv. Child Dev. Behav.*, **6**, 63 (1971).

<sup>5</sup> Winer, B. J., *Statistical Principles in Experimental Design*, 384 (McGraw-Hill, New York, 1971).

<sup>6</sup> Eimas, P. D., Siqueland, E., Jusczyk, P., and Vigorito, J., *Science*, **171**, 303 (1971).

<sup>7</sup> Suchman, R. G., and Trabasso, T., *J. exp. Child Psychol.*, **3**, 177 (1966).

## Sexual transfer of specific genes without gametic fusion

THE most common method of combining genes from two organisms is by sexual hybridisation. In plant breeding, however, a variety can often be improved considerably by introducing only one or a small number of specific genes. Elimination of accompanying undesirable characteristics normally requires several generations of backcrossing and selection which may greatly prolong the breeding period. Here I describe experimental results which suggest a possible new technique for more rapid transfer of desired genes between two organisms.

During experiments on the role of 'mentor pollen' in overcoming intraspecific incompatibility in *Nicotiana*, some unexpected observations were made. In some cases, as expected the use of 'killed' irradiated (100,000 rad, Co: 347.6 rad min<sup>-1</sup>) mentor pollen mixed with the viable maternal incompatible pollen led to the breakdown in the self incompatibility system resulting in the production of selfed progeny. (Mentor pollen is compatible irradiated pollen which produces a growth tube but fails to fertilise the ovule. In certain genotypic combinations substances are released by the mentor pollen which allow normally incompatible pollen to produce a growth tube able to fertilise the ovule.) In other cases the mentor pollen had no effect and usually no seed was produced. But in these latter combinations, which involved the related but morphologically distinct intercompatible species *N. forgetiana* and *N. alata*, in a few cases rare viable seeds were produced along with a large number of collapsed non-viable ovules.

These seeds gave rise to 24 normal, fertile, diploid plants which, in general morphological characteristics, all resembled their maternal parents. Surprisingly, however, most of these plants showed coloured flowers and/or an incompatibility allele characteristic of the mentor pollen source (Table 1). Fourteen of the 24 plants were examined cytologically and all were found to be normal. No additional chromosome fragments<sup>1-3</sup> were present.

To investigate this apparent 'gene transfer' phenomenon, 14 plants showing flower colour and/or an S allele typical of the mentor pollen source were crossed with various tester plants of known genotypes (Tables 2 and 3). The most significant result of these test crosses was the recovery of 34 plants carrying 3 S alleles from crosses C2-1, C2-2, C2-3, D6-1 and D7-1 (Table 3). Triallelic plants of this type cannot be obtained by any normal form of inheritance and their appearance is strong evidence for the occurrence of an unusual genetic transfer process.

A number of other anomalous features of these experimental results also suggest the transfer of genes from irradiated mentor pollen without normal gamete fusion:

(1) Although the exceptional plants may have been derived from rare pollen nuclei which survived the irradiation

Table 1 Mean looking times(s)

| Test wavelengths*<br>(nm) | Mean | S.d. |
|---------------------------|------|------|
| 450                       | 5.8  | 2.7  |
| 480                       | 5.7  | 2.8  |
| 510                       | 7.3  | 2.4  |

\*Luminances = 3.4 cd m<sup>-2</sup>.



**Table 1** Initial observations suggesting gene transfer in experiments involving the use of irradiated mentor pollen to overcome self incompatibility

| Family code | Cross                                                                            |                                                                          | Total no. of plants* | Progeny†                                                                                                     |                                                                                                                                                                                                                                                                                | Cytology†            |
|-------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------|--------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
|             | Selfed female parent<br><i>S</i> genotype and flower colour                      | Mentor pollen source<br><i>S</i> genotype and flower colour              |                      | No. of plants with stated<br><i>S</i> genotype and flower colour<br>(italics denote 'transferred' character) |                                                                                                                                                                                                                                                                                |                      |
| A           | <i>N. forgetiana</i><br><i>S</i> <sub>F1</sub> <i>S</i> <sub>F2</sub> White (rr) | <i>N. alata</i><br><i>S</i> <sub>3</sub> <i>S</i> <sub>3</sub> Red (Rr)  | 7<br>(A1 to A7)      | 4<br>2<br>1                                                                                                  | <i>S</i> <sub>F1</sub> <i>S</i> <sub>F1</sub> Red<br><i>S</i> <sub>F2</sub> <i>S</i> <sub>F2</sub> Red<br>?                                                                                                                                                                    | Normal<br>(3 plants) |
|             |                                                                                  |                                                                          |                      |                                                                                                              |                                                                                                                                                                                                                                                                                |                      |
| B           | as above                                                                         |                                                                          | 4<br>(B1 to B4)      | 1<br>1<br>2                                                                                                  | <i>S</i> <sub>F1</sub> <i>S</i> <sub>F1</sub> Red<br><i>S</i> <sub>F2</sub> <i>S</i> <sub>F2</sub> Red<br><i>S</i> <sub>F2</sub> <i>S</i> <sub>F2</sub> White                                                                                                                  | Normal<br>(3 plants) |
|             |                                                                                  |                                                                          |                      |                                                                                                              |                                                                                                                                                                                                                                                                                |                      |
| C           | <i>N. alata</i><br><i>S</i> <sub>F10</sub> <i>S</i> <sub>F11</sub> White (pp)    | <i>N. alata</i><br><i>S</i> <sub>1</sub> <i>S</i> <sub>3</sub> Pink (Pp) | 5<br>(C1 to C5)      | 1<br>1<br>1<br>1<br>1                                                                                        | <i>S</i> <sub>1</sub> <i>S</i> <sub>F10</sub> Pink<br><i>S</i> <sub>1</sub> <i>S</i> <sub>F11</sub> Pink<br><i>S</i> <sub>3</sub> <i>S</i> <sub>F11</sub> Pink<br><i>S</i> <sub>3</sub> <i>S</i> <sub>F11</sub> White<br><i>S</i> ? <i>S</i> <sub>F11</sub> White              | Normal<br>(3 plants) |
|             |                                                                                  |                                                                          |                      |                                                                                                              |                                                                                                                                                                                                                                                                                |                      |
| D           | as above                                                                         |                                                                          | 8<br>(D1 to D8)      | 1<br>2<br>1<br>3<br>1                                                                                        | <i>S</i> <sub>3</sub> <i>S</i> <sub>F11</sub> Pink<br><i>S</i> <sub>3</sub> <i>S</i> <sub>F11</sub> White<br><i>S</i> <sub>3</sub> <i>S</i> <sub>F10</sub> White<br><i>S</i> <sub>1</sub> <i>S</i> <sub>F10</sub> White<br><i>S</i> <sub>1</sub> <i>S</i> <sub>F11</sub> White | Normal<br>(5 plants) |
|             |                                                                                  |                                                                          |                      |                                                                                                              |                                                                                                                                                                                                                                                                                |                      |

\*Families were derived from single pods except for (B) which was derived from two pods with two seeds in each. Normally a pod contains up to 1,000 or more seeds.

†Only 14 of the 24 plants were examined cytologically.

‡To overcome the problem of breakdown of incompatibility reaction in parthenogenetic diploids when tested with highly heterozygous testers<sup>4</sup>, only appropriately inbred plants were used as *S* gene testers in these experiments.

treatment, the extremely high lethal dose of 100,000 rad has a margin of error which virtually rules out this possibility. In addition, any plant arising from fertilisation by an irradiated nucleus would be expected to show extreme sterility, lack of vigour, or gross morphological defects. No such effects were observed.

(2) In families A and B (Table 1) where the mentor pollen was obtained from a different species, the progeny were unequivocally maternal in floral and general morphology, and were definitely distinct from normal *F*<sub>1</sub> *N. forgetiana* × *N. alata* hybrids.

(3) In the same families, A and B, in which the mentor pollen parent was homozygous for the *S*<sub>3</sub> gene, the progeny were all homozygous for one or other maternal *S* allele, whereas if they arose through normal fertilisation by mentor pollen they would all have carried the *S*<sub>3</sub> allele. And yet the participation of the mentor pollen parent is unequivocal, for the red flower colour of the progeny could have come only from that parent.

(4) Also in families A and B, nine out of the 11 progeny were red-flowered, whereas according to the genotypes of the parents a true hybridisation should have given a ratio of 1 red to 1 white.

(5) Contamination with foreign pollen cannot explain the anomalous plants in Table 1. First, the plants were all grown in an insect-proof greenhouse and experimental procedures were strictly controlled. Second, contamination would be expected to produce extremely rare pods with a full set of viable seeds, but in these crosses the pods were too frequent and the number of viable seeds per pod too low to represent accidental contamination.

(6) Although the anomalous plants from crosses A to D are highly fertile (60–95% stainable pollen), some nevertheless show a greater degree of pollen sterility than is usually observed in normal cross-pollinated plants of this genus.

(7) Finally, a unique phenotypic abnormality was recovered among the progeny of four anomalous plants, C1, C2, C4 and D4. In these plants, which are otherwise normal in appearance, the 'floral' shoot became vegetative with tufts of small leaves in place of flower buds. When these plants were cut back to the stem base they regenerated the original

type of vegetative inflorescence. No such plants have previously been seen or reported in *Nicotiana*<sup>4</sup> to the author's knowledge. This novel phenotype was apparently generated as a consequence of unorthodox genetic recombination processes.

**Table 2** Segregation among the progeny of plants from crosses A and B which apparently carried transferred flower colour genes

| Family code | Cross*                                 |     | Red | Segregation |  | Expected† ratio |
|-------------|----------------------------------------|-----|-----|-------------|--|-----------------|
|             | (italics denote transferred character) |     |     | White       |  |                 |
| A2-1        | <i>A2 Red</i> × white                  | ♀ ♂ | 17  | 15          |  | 1:1             |
| 2           | [ <i>A2 Red</i> × white]               |     | 5   | 3           |  | 1:1             |
| 3           | [white × <i>A2 Red</i> ]               |     | 21  | 9           |  | 1:1             |
| A6-1        | <i>A6 Red</i> × white                  |     | 7   | 7           |  | 1:1             |
| 2           | [ <i>A6 Red</i> × white]               |     | 11  | 7           |  | 1:1             |
| 3           | [white × <i>A6 Red</i> ]               |     | 13  | 8           |  | 1:1             |
| 4           | <i>A6 Red</i> selfed                   |     | 11  | 2           |  | 3:1             |
| A7-1        | <i>A7 Red</i> × white                  |     | 7   | 6           |  | 1:1             |
| 2           | <i>A7 Red</i> × white                  |     | 3   | 7           |  | 1:1             |
| 3           | [ <i>A7 Red</i> × white]               |     | 13  | 15          |  | 1:1             |
| 4           | [white × <i>A7 Red</i> ]               |     | 9   | 5           |  | 1:1             |
| B1-1        | white × <i>B1 Red</i>                  |     | 18  | 15          |  | 1:1             |
| B4-1        | <i>B4 Red</i> × white                  |     | 24  | 16          |  | 1:1             |
| 2           | [ <i>B4 Red</i> × white]               |     | 19  | 17          |  | 1:1             |
| 3           | [white × <i>B4 Red</i> ]               |     | 19  | 7           |  | 1:1             |
| 4           | Red (Rr) × <i>B4 Red</i>               |     | 31‡ | 4           |  | 3:1             |
| 5           | <i>B4 Red</i> selfed                   |     | 29  | 4           |  | 3:1             |

\*Testers were normal diploid plants, homozygous recessive for flower colour (rr). In B4-4 a heterozygous red-flowered tester (Rr) was used. Brackets indicate reciprocal crosses.

†Segregation expected if the transferred gene is heterozygous and behaves normally. 1:1 segregations. Crosses A2-3 and B4-3 show significant excesses of red-flowered progeny ( $P < 0.05$ ). Overall there is a highly significant excess of red-flowered progeny ( $P < 0.01$ ). 3:1 segregations. Individual segregations are not significantly different from expectation, but the overall data show a highly significant excess of red-flowered progeny ( $P < 0.01$ ).

‡Includes normal and 'transferred' red flowered plants.

The following sequence of events is proposed as a working hypothesis to explain the above results.

The extremely high dose of irradiation given to the mentor pollen 'pulverises' the generative nucleus to produce a mass of fine chromatin fragments. Division of the generative nucleus fails, and this may contribute further to chromatin fragmentation. Discharge of the pulverised chromatin from the pollen tube into the egg acts as a 'pseudo-fertilisation'. The block to cell division in the egg is lifted as usual and the chromosomes are replicated, but the presence of disorganised pollen chromatin prevents a normal first zygotic division. This results in a diploid egg with the 'induced' physiology of a zygote. Presumably, at least in certain cells, the free chromatin fragments are rapidly degraded, or lost, and their disorganising effects are soon overcome to enable subsequent mitotic divisions to occur normally. The resulting embryo will be a parthenogenetic diploid. Rare occurrence of parthenogenetic diploidy after pollination with irradiated pollen has previously been reported in *Nicotiana*<sup>3</sup>. The slightly greater pollen sterility shown by some of the anomalous plants is mainly attributed to parthenogenetic diploidy exposing deleterious recessive genes.

Fragments of mentor pollen chromatin may occasionally associate with their homologues among the egg chromatin. These fragments will have broken 'sticky' ends and may insert into nicks formed during chromosome replication<sup>6</sup>. The following possibility is suggested to explain why the pollen chromatin fragments should be attracted to the homologous segments in the egg chromatin in a manner reminiscent of meiotic pairing. Once replication has been induced, the homologous pollen chromatin molecules are

able to compete successfully with the *de novo* synthesised molecules, since they occupy the respective positions next to the strand before the appropriate molecules could be freshly synthesised.

Once chromatin segments from pollen have paired with those of the egg, substitution or addition may easily occur during replication. Three types of chromatin transfer can be imagined (Fig. 1a, b, c). It is interesting to note that results consistent with these patterns of transfer have actually been found (Table 3). Plant D6 illustrates the triallelic state (Fig. 1b), and plant C2 probably illustrates the alternative triallelic state (Fig. 1c). Plants C2 and D7—the third plant showing the triallelic state—illustrates the instability of the exosome, producing three types of gametes (Fig. 1e). The remainder of tested plants in Table 3 represent the substituted state (Fig. 1a).

The gene transfers observed in this study seem to be fairly stable. No variegated or blotchy flower coloration, characteristic of *Petunia* transformations<sup>7</sup>, was obtained. Only in certain rare cases are there noteworthy deviations from expected segregation ratios (Table 3). As regards flower colour, however, there may be a general tendency to recover an excess of coloured flowered plants bearing the transferred gene. This apparent selection in favour of the transferred gene is not related to the direction of the cross, thus ruling out any advantage in terms of pollen tube growth.

Previous 'transformation' studies<sup>7-10</sup> with higher organisms have so far been unable to distinguish unequivocally between substitution and addition models of chromatin transfer. In the present results, since the presence or absence of individual *S* alleles in the style can be determined

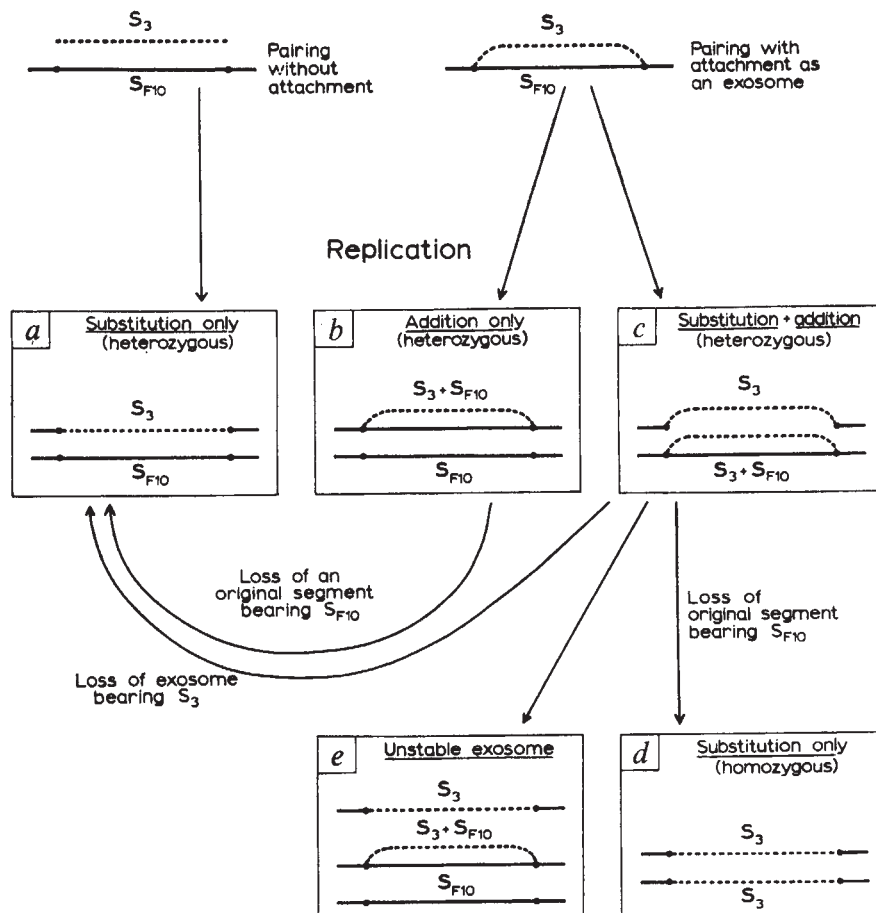


Fig. 1 Diagrammatic illustration of proposed patterns of gene transfer. Genetic states corresponding to a, b, c and e have been observed experimentally (see text). — Egg chromatin strand (or replicated copy). - - - - - Irradiated pollen chromatin (or replicated copy). Single lines represent strands of 'chromatin', not single strands of DNA.

**Table 3** Segregation among the progeny of plants from crosses C and D which apparently carried transferred flower colour and/or *S*-genes

| Family code |    | Cross*                                                  |                                             |                                              |                                                        | Segregation                                                |            |           |  | Flower colour |       |           |
|-------------|----|---------------------------------------------------------|---------------------------------------------|----------------------------------------------|--------------------------------------------------------|------------------------------------------------------------|------------|-----------|--|---------------|-------|-----------|
|             |    | (italics denote transferred character)                  |                                             |                                              |                                                        | S genotypes                                                | Triallelic | Expected† |  | Pink          | White | Expected‡ |
| C1          | —1 | C1( <i>S<sub>3</sub>S<sub>F11</sub></i> , <i>Pink</i> ) | × <i>S<sub>1</sub>S<sub>1</sub></i> Pp      | 10 ( <i>S<sub>1</sub>S<sub>3</sub></i> )§§   | 21 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             |                                                            |            | 1:1       |  | 25§           | 12    | 3:1       |
|             | 2  | C1                                                      | × <i>S<sub>F10</sub>S<sub>F11</sub></i> pp  | 13 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )   | 12 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )           |                                                            |            | 1:1       |  | 16            | 11    | 1:1       |
|             | 3  | <i>S<sub>F10</sub>S<sub>F11</sub></i> pp                | × C1                                        | 13 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )   | 9 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )              |                                                            |            | 1:1       |  | 16            | 14    | 1:1       |
| C2¶         | —1 | C2( <i>S<sub>1</sub>S<sub>F11</sub></i> , <i>Pink</i> ) | × <i>S<sub>F10</sub>S<sub>F11</sub></i> pp  | 13 ( <i>S<sub>1</sub>S<sub>F10</sub></i> )   | 10 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )           | 5 ( <i>S<sub>1</sub>S<sub>F10</sub>S<sub>F11</sub></i> )   |            |           |  | 16            | 14    | 1:1       |
|             | 2  | <i>S<sub>F10</sub>S<sub>F11</sub></i> pp                | × C2                                        | 9 ( <i>S<sub>1</sub>S<sub>F10</sub></i> )    | 13 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             | 9 ( <i>S<sub>1</sub>S<sub>F10</sub>S<sub>F11</sub></i> )   |            |           |  | 24            | 8††   | 1:1       |
|             | 3  | C2                                                      | × <i>S<sub>3</sub>S<sub>3</sub></i> Pp      | 19 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )   | 8 ( <i>S<sub>1</sub>S<sub>3</sub>S<sub>F11</sub></i> ) | 8 ( <i>S<sub>1</sub>S<sub>3</sub>S<sub>F11</sub></i> )     |            |           |  | 15§           | 15‡‡  | 3:1       |
|             | 4  | C2                                                      | selfed                                      | 4 ( <i>S<sub>1</sub>S<sub>1</sub></i> )      | 17 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             | 3 ( <i>S<sub>F11</sub>S<sub>F11</sub></i> )                |            |           |  | 23            | 3     | 3:1       |
| C4†         | —1 | C4( <i>S<sub>3</sub>S<sub>F11</sub></i> )               | × <i>S<sub>1</sub>S<sub>1</sub></i>         | 8 ( <i>S<sub>1</sub>S<sub>3</sub></i> )      | 6 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )              |                                                            |            | 1:1       |  |               |       |           |
|             | 2  | <i>S<sub>F10</sub>S<sub>F11</sub></i>                   | × C4                                        | 4 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )    | 2 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )              |                                                            |            | 1:1       |  |               |       |           |
| D1          | —1 | D1( <i>S<sub>3</sub>S<sub>F11</sub></i> , <i>Pink</i> ) | × <i>S<sub>F10</sub>S<sub>F11</sub></i> pp  | 9 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )    | 10 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )           |                                                            |            | 1:1       |  | 11            | 8     | 1:1       |
|             | 2  | D1                                                      | × <i>S<sub>1</sub>S<sub>1</sub></i> Pp      | 15 ( <i>S<sub>1</sub>S<sub>3</sub></i> )     | 13 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             |                                                            |            | 1:1       |  | 25§           | 9     | 3:1       |
|             | 3  | <i>S<sub>F10</sub>S<sub>F11</sub></i> pp                | × D1                                        | 3 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )    | 12 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )¶¶           |                                                            |            | 1:1       |  | 10            | 6     | 1:1       |
|             | 4  | D1                                                      | selfed                                      | 7 ( <i>S<sub>3</sub>S<sub>3</sub></i> )      | 13 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )             | 6 ( <i>S<sub>F11</sub>S<sub>F11</sub></i> )                |            | 1:2:1     |  | 25            | 5     | 3:1       |
| D2†         | —1 | D2( <i>S<sub>3</sub>S<sub>F11</sub></i> )               | × <i>S<sub>1</sub>S<sub>1</sub></i>         | 13 ( <i>S<sub>1</sub>S<sub>3</sub></i> )     | 15 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             |                                                            |            | 1:1       |  |               |       |           |
|             | 2  | D2                                                      | × <i>S<sub>F10</sub>S<sub>F11</sub></i>     | 12 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )   | 9 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )            |                                                            |            | 1:1       |  |               |       |           |
|             | 3  | <i>S<sub>F10</sub>S<sub>F11</sub></i>                   | × D2                                        | 14 ( <i>S<sub>3</sub>S<sub>F10</sub></i> )   | 12 ( <i>S<sub>3</sub>S<sub>F11</sub></i> )             |                                                            |            | 1:1       |  |               |       |           |
| D4†         | —1 | <i>S<sub>F10</sub>S<sub>F11</sub></i>                   | × D4( <i>S<sub>1</sub>S<sub>F11</sub></i> ) | 5 ( <i>S<sub>1</sub>S<sub>F10</sub></i> )    | 12 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )             |                                                            |            | 1:1       |  |               |       |           |
| D5†         | —1 | D5( <i>S<sub>1</sub>S<sub>F10</sub></i> )               | × <i>S<sub>F10</sub>S<sub>F11</sub></i>     | 18 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )§§ | 0 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )            |                                                            |            | 1:1       |  |               |       |           |
| D6†         | —1 | D6( <i>S<sub>3</sub>S<sub>F11</sub></i> )               | × <i>S<sub>1</sub>S<sub>1</sub></i>         | 13 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )   |                                                        | 8 ( <i>S<sub>1</sub>S<sub>3</sub>S<sub>F11</sub></i> )     |            |           |  |               |       |           |
| D7†         | —1 | D7( <i>S<sub>1</sub>S<sub>F10</sub></i> )               | × <i>S<sub>F11</sub>S<sub>F11</sub></i>     | 11 ( <i>S<sub>1</sub>S<sub>F11</sub></i> )   | 3 ( <i>S<sub>F10</sub>S<sub>F11</sub></i> )            | 4 ( <i>S<sub>1</sub>S<sub>F10</sub>S<sub>F11</sub></i> )** |            |           |  |               |       |           |

\*Testers were normal diploid plants with white (pp) or pink (Pp) flowers. Brackets indicate reciprocal crosses.

†These are white flowered plants with no apparent 'transferred' flower colour factors.

‡Segregation expected if the transferred gene is heterozygous and behaves normally.

§Includes normal and 'transferred' red flowered plants.

¶Plant C2(*S<sub>1</sub>S<sub>F11</sub>*) apparently forms (*S<sub>1</sub>S<sub>F11</sub>*) as well as (*S<sub>1</sub>*) and (*S<sub>F11</sub>*) gametes and viability of different types of gametes vary between combinations.

||Plant D6(*S<sub>3</sub>S<sub>F11</sub>*) apparently forms gametes (*S<sub>F11</sub>*) and (*S<sub>3</sub>S<sub>F11</sub>*) only.

\*\*Plant D7(*S<sub>1</sub>S<sub>F10</sub>*) apparently forms (*S<sub>1</sub>S<sub>F10</sub>*) as well as (*S<sub>1</sub>*) and (*S<sub>F10</sub>*) gametes.

††Highly significant excess of pink-flowered plants ( $P < 0.01$ ). All other '1:1' segregations do not differ significantly from expectation but are homogeneous in direction. The overall data show a highly significant excess of pink-flowered progeny ( $P < 0.01$ ).

‡‡Highly significant deficit of pink-flowered plants ( $P < 0.01$ ). All other '3:1' segregations do not differ significantly from expectation.

§§One class carrying transferred *S* gene. Cross C1-1 shows a significant deficit of the transferred gene ( $P < 0.05$ ). Cross D5-1 shows a highly significant excess of the transferred gene ( $P < 0.01$ ). Other segregations do not differ significantly from expectation.

¶¶Both classes carrying the transferred *S* gene. Cross D1-3 shows a significant excess of the *S<sub>3</sub>S<sub>F11</sub>* combination. All other segregations do not differ significantly from expectation.

|||Contained certain number of abnormal plants with vegetative floral shoots (C<sub>1/3</sub>—5 plants, C<sub>2/2</sub>—8, C<sub>2/4</sub>—2, C<sub>4/2</sub>—2, D<sub>4/1</sub>—3).

it has been possible to demonstrate that both substitution and addition have occurred, although substitution seems to be predominant. It should be noted, however, that the patterns of transfer seen in these experiments may not be strictly comparable with those seen in previous attempts to induce transformation in higher organisms with chemically extracted preparations of DNA and chromatin. Extraction procedures and consequent denaturation of chromatin<sup>11</sup> are avoided by the present method, and the exogenous chromatin is received by a single specialised type of cell.

There are certain important features of the present results for which there seems to be no reasonable explanation at present. The frequency of specific transfers of the flower colour and *S* genes (the only two genes investigated) is unaccountably high among the rare seeds produced. One might suggest that there is a selection for the *S* gene transfer, for the heterozygosity of the *S* complex which is involved in the physiology of reproduction may be instrumental in the induction of parthenogenetic diploidy. The same cannot be said to be true of the flower colour gene however. There is a lack of *S* gene transfers in families A and B (Table 1) compared with families C and D. The segregation data, particularly of plants C1, C2, D1, D5 and D7, suggest the existence of situations which may influence gametic selection in extreme and contradictory ways. Apparently, wide variability is inherent in this induced phenomenon.

Doy *et al.*<sup>12</sup> have coined the term 'transgenesis' to describe the "transfer of genetic information from one cell to

another followed by phenotypic expression". The present phenomenon in *Nicotiana* can be regarded as a specialised form of 'sexual transgenesis'. Although there are certain mysterious aspects of the gene transfer phenomenon observed in these experiments, these new techniques are theoretically and technically attractive.

I thank Mr R. J. Trott, Palmerston North Hospital, for his help in the irradiation of pollen and Mr G. B. Petterson for technical assistance.

K. K. PANDEY

Genetics Unit,  
Grasslands Division, DSIR,  
Palmerston North,  
New Zealand

Received February 17; accepted June 2, 1975.

- Brewbaker, J. L., and Natarajan, A. T., *Genetics*, **45**, 699–704 (1960).
- Pandey, K. K., *Nature*, **206**, 792–795 (1965).
- Pandey, K. K., *Heredity*, **22**, 255–284 (1967).
- Goodspeed, T. H., *The Genus Nicotiana* (Chronica Botanica, Waltham, Massachusetts, 1954).
- Pandey, K. K., *Theoret. appl. Genet.*, **44**, 199–205 (1974).
- De Petrocellis, B., and Monroy, A., *Endavour*, **33**, 92–97 (1974).
- Hess, D., *Z. Pflanzenphysiol.*, **60**, 348–358 (1969).
- Fox, A. S., Yoon, S. B., Duggleby, W. F., and Gelbart, W. M., in *Informative Molecules in Biological Systems* (edit. by Ledoux, L.), 313–326 (North Holland, Amsterdam, 1971).
- Ledoux, L., Huart, R., and Jacobs, M., *Nature*, **249**, 17–21 (1974).
- Hess, D., *Z. Pflanzenphysiol.*, **68**, 432–440 (1973).
- Merrill, C. R., and Stanbro, H., *Z. Pflanzenphysiol.*, **72**, 371–388 (1974).
- Doy, C. H., Gresshoff, P. M., and Rolfe, B. G., in *The Biochemistry of Gene Expression in Higher Organisms* (edit. by Pollak, J. K., and Lee, J. W.), 21–37 (Australian and New Zealand Book Co., Sydney, 1973).



## Salt-dependent bacteriophage infecting *Halobacterium cutirubrum* and *H. halobium*

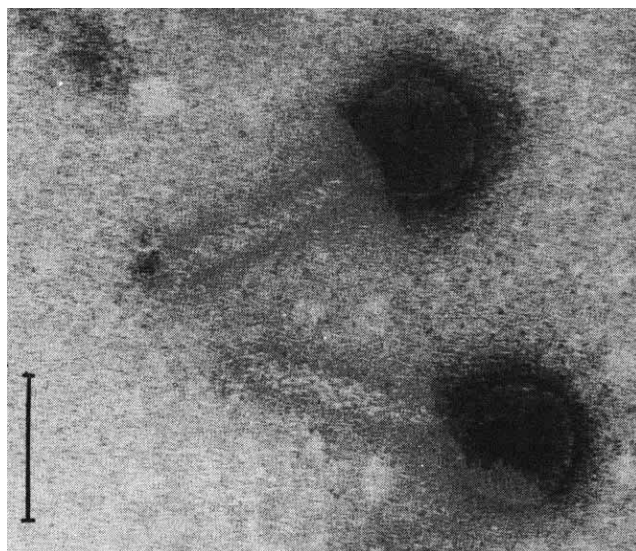
MOST molecular genetic mechanisms are sensitive to ionic strength. The isolation of phage for *Halobacterium* allows a study of genetic adaptation in the extremes of ionic strength. The usual requirement for low ion concentrations is reversed in *Halobacterium* which maintains intracellular KCl near saturation<sup>1</sup>. As a result, macromolecular structure may be altered sufficiently to favour genetic mechanisms that occur rarely or not at all in other organisms. The presence of a unique DNA density distribution<sup>2</sup> and RNA-dependent RNA-polymerase<sup>3</sup> in *Halobacterium* suggests that such alterations occur.

Specific ion requirements define, to a great extent, the ecological niche of halophilic species, providing an advantage in general studies of phage ecology. The nature of halophilic genetic adaptation has a bearing on the suitability of seawater evaporates as sites for biogenesis and evolution.

*Halobacterium* flourishes in salt saturated environments and requires 10–12% NaCl to remain viable<sup>4</sup>. Water samples bearing phage were collected from The Salt Ponds of Yallahs, Jamaica, where the salt concentration varies from about 10% to saturation, depending on rainfall. Enrichment cultures were prepared from 1 ml of crude sample incubated in halophile broth for five d at 37 °C with aeration followed by addition of 2 ml late stationary phase *H. cutirubrum* and 10 d of additional incubation. The resulting culture was shaken with 1 ml chloroform and the supernatant collected after low speed centrifugation. Phages were purified by picking isolated plaques several times in succession from plates showing only a single plaque with *H. cutirubrum* as indicator.

Only phages forming clear plaques were detected in enrichment cultures or by direct plating of a sample containing 20% salt collected when the halobacterial flora was returning after having been greatly reduced by rainwater. One of these was selected and designated Halophage Ja. 1. The host range of phage Ja. 1 includes strains of *H. cutirubrum* and *H. halobium* reported in Table 1. The frequency of colonies surviving high multiplicities of phage varies widely among sensitive strains and several resistant colonies showed altered pigmentation. A mixed population of phages forming clear and turbid plaques on *H. cutirubrum* was present in a second sample collected 5 months later after evaporation had resulted in salt saturation and a red halobacterial bloom.

For determination of nucleic acid content lysates of Ja. 1 were purified by differential centrifugation and filtration through Whatman No. 1 filters. Concentrated suspensions were dialysed into 0.2 M NaCl, 0.05 M MgSO<sub>4</sub>, 0.01 M Tris (pH 7.4) before a final cycle of low and high speed centrifugation. This procedure removes nucleic acid adhering to heavy cell debris while phage viability is maintained. Purified suspensions containing 10<sup>12</sup> phage per ml were dialysed into 0.1 M NaCl, producing an extremely viscous clear solution of disrupted phage and <10<sup>4</sup> viable phage per ml. Reaction of the disrupted phage with diphenylamine<sup>5</sup> showed 1.5 × 10<sup>8</sup> D DNA are present for each viable unit before disruption. This amount of DNA slightly exceeds the estimated capacity of the phage head shown in Fig. 1. Further purification of phage Ja. 1 on preformed CsCl gradients gave a single, sharp band of viability at 1.55 g cm<sup>-3</sup> containing proportionately the same amount of DNA per viable unit. The presence of double-stranded DNA is confirmed by specific immunological assay as is single stranded DNA after boiling for 5 min. Double



**Fig. 1** Halophage Ja.1 suspensions in 0.2 M NaCl, 0.05 M MgSO<sub>4</sub> were fixed on Formvar-carbon-coated grids in glutaraldehyde vapours for 10 s and negatively stained with uranyl acetate. The bar represents a distance of 0.1 μm. The phages pictured here are of the same morphological group although larger than particles observed in crude preparations of *H. salinarum* flagella<sup>11</sup>.

stranded RNA, RNA-DNA hybrids and single stranded DNA before heat treatment are not detected with corresponding antisera (refs 6 and 7 and A.C.W. and B.D.S., unpublished).

**Table 1** Host range of Ja.1

| Host organism                 | Source            | Plating efficiency    |
|-------------------------------|-------------------|-----------------------|
| 1. <i>H. cutirubrum</i>       | Laboratory strain | 1.0                   |
| 2. <i>H. cutirubrum</i>       | S. T. Bayley      | 0.2                   |
| 3. <i>H. cutirubrum</i>       | L. I. Hochstein   | <2 × 10 <sup>-9</sup> |
| 4. <i>H. halobium</i> , Brown | L. I. Hochstein   | 0.6                   |
| 5. <i>H. halobium</i> , Delft | L. I. Hochstein   | 0.01                  |
| 6. <i>H. halobium</i> , Delft | K. Eimhjellen     | <2 × 10 <sup>-9</sup> |
| 7. Utah 18*                   | L. I. Hochstein   | 0.4                   |
| 8. Utah*                      | L. I. Hochstein   | <2 × 10 <sup>-9</sup> |
| 9. AR1, Lanyi†                | L. I. Hochstein   | <2 × 10 <sup>-9</sup> |
| 10. <i>Sarcina morrhuae</i>   | L. I. Hochstein   | <2 × 10 <sup>-9</sup> |
| 11. <i>Sarcina littoralis</i> | K. Eimhjellen     | <2 × 10 <sup>-9</sup> |
| 12. <i>H. salinarum</i>       | K. Eimhjellen     | <2 × 10 <sup>-9</sup> |

Host organisms were grown at 37 °C with shaking in halophile broth containing 9 parts salt solution (20% NaCl, 2% KCl, 2% MgSO<sub>4</sub>), and 1 part nutrient solution (4% Difco yeast extract, 4% Difco casamino acids, 3% sodium citrate, 20% NaCl, 2% KCl, 2% MgSO<sub>4</sub>). Agar for agar plates was prepared by mixing 500 ml agar salts solution (25% NaCl, 2.5% KCl, 2.5% MgSO<sub>4</sub>), 60 ml nutrient solution and 8 g Difco agar in 100 ml distilled water. All solutions were autoclaved at 121 °C before use. Agar for overlays was prepared by mixing 40 ml salt solution, 50 ml distilled water, 0.4 g ion agar No. 2 and autoclaving for 15 min at 121 °C. 10 ml nutrient solution was added to the hot solution and 2.5-ml aliquots dispensed in small tubes. These were allowed to solidify, melted and tempered to 60 °C as needed. Host bacteria were collected by centrifugation in mid log phase and concentrated 20 times in a medium containing 9 parts agar salts solution and 1 part nutrient solution and held at 0–4 °C. Five drops of the host suspension was added to the overlay before the addition of phage. Plates were incubated at 38 °C and plaques appeared in less than 24 h. Lysates of phage Ja.1 containing 2–3 × 10<sup>10</sup> phage ml<sup>-1</sup> were produced by diluting 30 ml of a late stationary phase culture of *H. cutirubrum* into 200 ml halophile medium, infecting with eight 25-h plaques and shaking vigorously, until lysis was complete, about 24 h at 37 °C. Phage dilutions were made into complete halophile medium or a similar high salt nutrient solution.

\*Isolated from Great Salt Lake, USA.

†Isolated from salterns near San Francisco, USA.

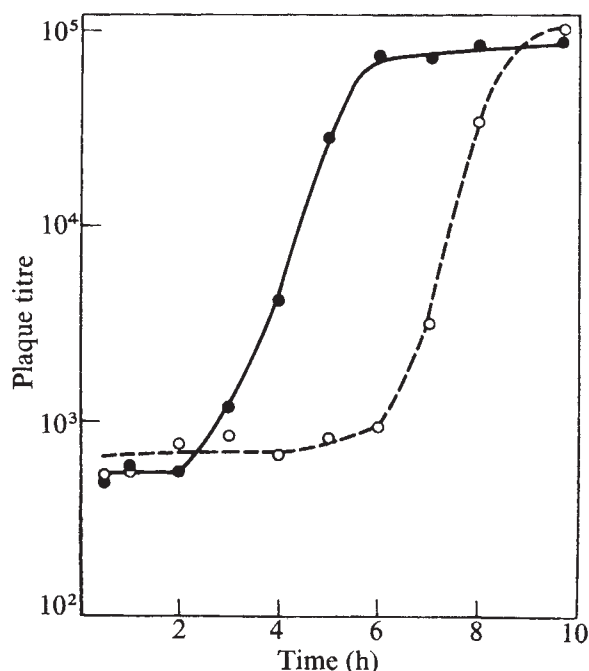
Table 2 Phage Ja.1 survival in ionic solutions

| Solution                             | Fraction surviving |                  |                    |
|--------------------------------------|--------------------|------------------|--------------------|
|                                      | 30min              | 24h              | 96h                |
| 3.5 M NaCl, 0.05 M MgSO <sub>4</sub> | 1.0                | 1.0              | 1.0                |
| 2 M NaCl                             | 0.8                | 0.6              | 0.3                |
| 1 M NaCl                             | 0.5                | 10 <sup>-6</sup> | < 10 <sup>-7</sup> |
| 0.2 M NaCl                           | < 10 <sup>-7</sup> | —                | —                  |
| 2 M KCl                              | 0.8                | 0.6              | 0.5                |
| 1 M KCl                              | 0.7                | 0.1              | 0.01               |
| 0.2 M KCl                            | < 10 <sup>-7</sup> | —                | —                  |
| 0.02 M MgSO <sub>4</sub>             | 0.7                | 0.6              | 0.4                |

A plate stock containing  $2 \times 10^{10}$  phage Ja. 1 ml<sup>-1</sup> diluted 1:200 into various ionic solutions at 4 °C each containing 0.01 M Tris buffer (pH 7.4). Phage titres were determined at intervals after dilution. Relative rates of inactivation are similar at 37 °C.

The single step growth function for phage Ja. 1 infection of *H. cutirubrum* is shown in Fig. 2. Only 25% of the input phage are present as chloroform sensitive infective centres after a 30-min adsorption period and 25% are present as chloroform resistant, presumably unadsorbed particles. After a 2-h eclipse, maturation of phage begins and is complete in 6 h which is the normal generation time for the bacteria alone growing in the same conditions. The apparent average burst size is 140. Lysis begins only after maturation is complete and occurs over a 3–4-h period. The abrupt cessation of phage maturation before lysis suggests that a specific function limits the number of particles produced in each cell. Phage T4, a model of lytic development, continues to mature particles in an infected cell until lysis is achieved<sup>8</sup>. Inefficient adsorption, limited maturation, and delayed lysis in Ja. 1 infection may favour an equilibrium between phage and host populations in a closed natural habitat. These

Fig. 2 A *H. cutirubrum* culture in log phase containing  $1.5 \times 10^8$  viable cells ml<sup>-1</sup> was infected with  $1.2 \times 10^7$  plaque forming units ml<sup>-1</sup> from a plate stock of Ja.1. After a 30-min adsorption period the culture was diluted times  $6 \times 10^3$  into fresh medium at 37 °C. Aliquots of the diluted culture were titred at intervals before and after adding a drop of chloroform. Chloroform lyses the cells but does not reduce the titre of completed phage particles. Plaque titre in the absence of chloroform is plotted minus the number of complete, chloroform resistant, particles present at early times to derive the titre of cells producing phage plus the titre of phage released from these in the culture, ○. The titres obtained after chloroform treatment, representing all complete phage present is plotted, ●.



functions could not benefit T4 whose host, *Escherichia coli*, cannot establish a stable population outside the gut.

Viability in phage Ja. 1 is preserved only in solutions of high ionic strength. The ions and concentrations required to protect Ja. 1 reflect intracellular ionic conditions in *H. cutirubrum* and are similar to those that favour the stability of halophilic enzymes<sup>9</sup>. With few exceptions, enzymes isolated from *Halobacterium* require 1 M to 4 M concentrations of monovalent cations for optimum stability and activity. In most instances K<sup>+</sup> is more efficient than Na<sup>+</sup> in filling this requirement and 0.05 M Mg<sup>2+</sup> often reduces or eliminates a requirement for monovalent ions. Table 2 shows that 1 M KCl affords Ja. 1 several orders of magnitude greater protection against irreversible loss of viability than does 1 M NaCl and a high degree of stability is conferred by 0.02 M Mg<sup>2+</sup>. In contrast, host viability and growth is strictly dependent on Na<sup>+</sup> concentrations exceeding 2 M and the presence of other ions does not materially reduce this dependence<sup>1</sup>. Phage survival in 0.02 M Mg<sup>2+</sup> suggests that the phage survives and may even function in more moderate environments than does its host species. At the opposite extreme, 10<sup>8</sup> phage Ja. 1 are destroyed by drying for 2 d at 22 °C and moderate humidity although the host species survives for many years in salt crystals<sup>10</sup>.

Contrary to the finding for Ja. 1, marine phage<sup>12</sup> survives better in NaCl than in KCl although marine bacteria often have high intracellular KCl<sup>1</sup>. Sparse host populations in the marine environment may cause phage to adapt to extracellular conditions while seawater evaporates, such as The Salt Ponds of Yallahs, support extremely dense microbial populations. Further study of the sequence of appearance, ionic niche requirements, and cellular genetic roles of halophages may reveal their ecological function in the environmentally induced growth cycles of the halophilic flora.

We thank Gary Inwald for technical assistance, the Department of Genetics and Plant Science for providing the electron microscope facilities, and Dr E. B. Goldberg for help.

A. C. WAIS\*

M. KON

R. E. MACDONALD

Section of Biochemistry, Molecular and Cell Biology,  
Cornell University,  
Ithaca, New York

B. D. STOLLAR

Tufts University School of Medicine,  
Boston, Massachusetts

Received April 10; accepted June 2, 1975.

\*To whom correspondence should be sent.

- Christian, J. H. B., and Waltho, J. A., *Biochim. biophys. Acta*, **65**, 506–508 (1962).
- Moore, R. L., and McCarthy, B. J., *J. Bact.*, **99**, 248–254; 255–262 (1969).
- Louis, B. G., and Fitt, P. S., *Biochem. J.*, **128**, 755–762 (1972).
- Larsen, H., *Adv. Microbiol. Physiol.*, **1**, 39–90 (1967).
- Burton, K., in *Methods in Enzymology*, **12**, Part B (edit. by Grossman, L., and Moldave, K.), 163–165 (Academic, New York and London, 1968).
- Stollar, B. D., *Science*, **169**, 609–611 (1970).
- Stollar, B. D., and Sandberg, A. L., *J. Immunol.*, **96**, 755–763 (1966).
- Wais, A. C., and Goldberg, E. B., *Virology*, **55**, 397–399 (1973).
- Lanyi, Y. K., *Bact. Rev.*, **38**, 272–290 (1974).
- Larsen, H., in *The Bacteria*, 4 (edit. by Gunsalus, I. C., and Stanier, R. Y.), 279–342 (Academic, New York and London, 1962).
- Torsvic, T., and Dundas, I. D., *Nature*, **248**, 680–681 (1974).
- Keynan, A., Neelson, K., Sideropoulos, H., and Hastings, J. W., *J. Virol.*, **14**, 333–340 (1974).

## Morphology and biochemistry of small, intensely fluorescent cells of sympathetic ganglia

NEURAL transmission in sympathetic ganglia has been regarded as an automatic relay involving only pre- and postganglionic neurones. It is now agreed, however, that the small, intensely fluorescent cells — at least some of



which are interneurons<sup>1,2</sup> — modulate transmission in the superior cervical ganglion. Greengard *et al.*<sup>3-6</sup> have documented the role of the ganglionic interneurone in modulating ganglionic transmission. Dopamine released from an interneurone binds to a receptor on the surface of the ganglionic neurone, activating an adenylate cyclase. The increased production of cyclic AMP leads to hyperpolarisation of the ganglionic neurone by a mechanism which is still obscure.

Studies (T.C. and T.H.W., unpublished) have shown that the morphology of the sympathetic interneurone is considerably more complex and shows more species diversity than is sometimes appreciated, which raises the possibility of major species differences in its biochemistry and in the mechanisms whereby it modulates transmission in the ganglion. We have demonstrated that the biochemistry of the interaction between the ganglionic interneurone and the principal ganglionic neurone is different in the cat from that in the cow.

Figure 1 shows the effect of dopamine on the production of cyclic AMP in bovine and feline superior cervical ganglia incubated *in vitro*. In the bovine ganglion there is a marked increase in the amount of cyclic AMP produced by even a 1  $\mu$ M concentration of dopamine and, moreover, the response obtained is proportional to log dopamine concentration (is dose dependent<sup>7</sup>). Our work in the bovine ganglion confirms the findings of Kebabian and Greengard<sup>8</sup>. In the feline ganglion (Fig. 1) the situation is different. The cat ganglion proved relatively unresponsive to dopamine stimulation and, within the range of concentrations used, the cyclic AMP response was not dose dependent. We also determined the effect of *l*-noradrenaline on the cat superior cervical ganglion incubated *in vitro* to make certain that we were not attempting to stimulate with the incorrect neuro-transmitter. This compound was ineffective even at concentrations of 100  $\mu$ mol l<sup>-1</sup> (ref. 9). Thus, the feline ganglion contains an adenylate cyclase which is relatively unresponsive to stimulation by dopamine or *l*-noradrenaline.

These findings led us to search for a morphological basis for these biochemical differences. Do the ganglionic interneurons of cat superior cervical ganglion differ from those of the bovine ganglion in a manner which could account for the differences in the observed responses to dopamine? Morphological studies of bovine and feline superior cervical ganglia were undertaken using the method of Grillo *et al.*<sup>10</sup>, as modified by T.C. and T.H.W. (unpublished). This method enables the preparation of sections for fluorescence microscopy and subsequent examination of the same material by electron microscopy. In both species two types

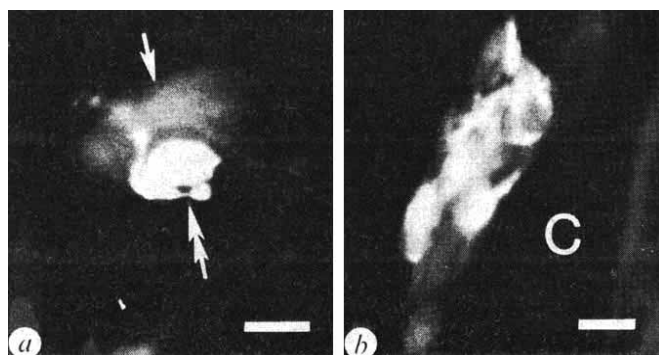


Fig. 2 Histochemical preparations demonstrating catecholamine fluorescence. *a*, Single small intensely fluorescent cell (type I) is seen in close apposition to a ganglionic neurone (bovine superior cervical ganglion). Single arrow, a principal ganglionic neurone; double arrow, a ganglionic interneurone with a process. *b*, Cluster of small fluorescent cells (type II) in the capsular connective tissue of the feline superior cervical ganglion. *c*, Capillary lumen. Bar represents 20  $\mu$ m.

of small, intensely fluorescent cells were distinguished. Type I (Fig. 2*a*) has long processes which run in close apposition to ganglion cells. This is regarded as the type of cell shown by Williams<sup>1,2</sup> to be an interneurone in the rat: the presence of this type I ganglionic interneurone is crucial to the Greengard hypothesis. The type II cell (Fig. 2*b*) is located in the interstitial or subcapsular portions of

Fig. 3 Ultrastructure of type II small, intensely fluorescent cells in cat and cow. *a*, Cat (perfusion-fixed for fluorescence microscopy and electron microscopy) showing characteristic dense granules (1,400–1,800 Å). *b*, Cow (immersion-fixed) also exhibiting dense granules. Hollow centres of these granules are presumed to be a fixation artefact. Bar represents 1  $\mu$ m.

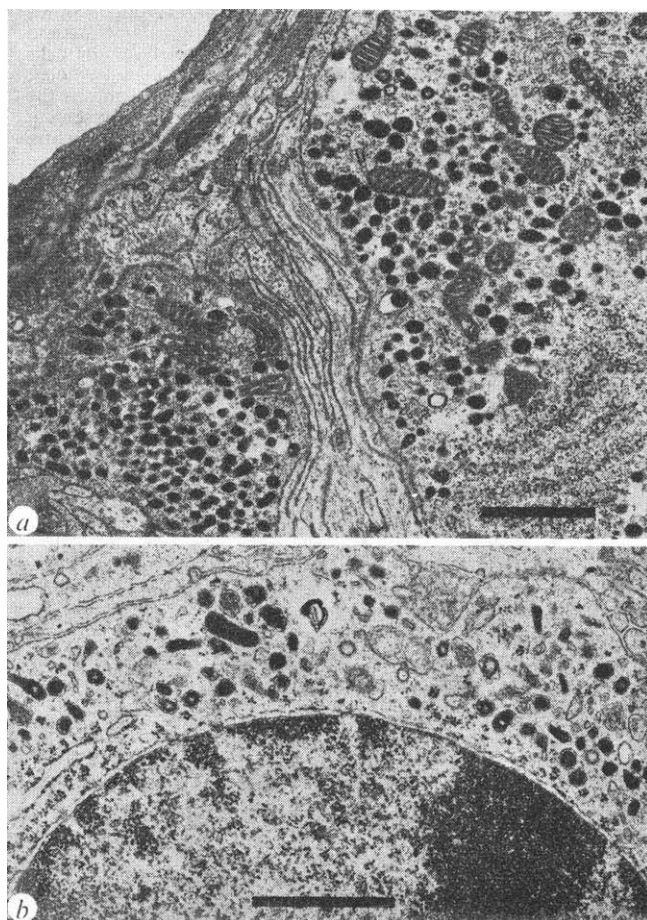
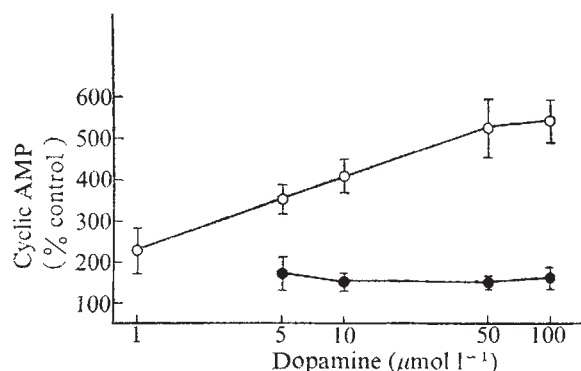


Fig. 1 Effect of dopamine on cyclic AMP levels of superior cervical ganglia incubated *in vitro* at 37 °C. ○, Bovine ganglia; ●, feline ganglia. Control values: bovine ganglia 28.8 ± 2.18 pmol cyclic AMP per mg protein; feline ganglia 26.9 ± 5.7 pmol per mg protein. Each point is the mean of four to six samples ± s.e. mean. All samples were incubated in Eagle's MEM containing 1 mM theophylline. Cyclic AMP assays were carried out according to Gilman<sup>12</sup>.





the ganglion, and has shorter processes which end in close relation to blood vessels<sup>11</sup>. Type II cells from both feline and bovine superior cervical ganglia exhibited similar ultrastructural features (Fig. 3a and b).

Quantitative studies of the total small, intensely fluorescent cell population in each species, using fluorescence histochemistry, showed that the bovine superior cervical ganglion contains a total population of 6,480 small, intensely fluorescent cells per ganglion (5.2 cells per mg wet tissue), and the feline ganglion contains 104 cells (4.1 cells per mg wet tissue). Thus the numbers per mg tissue are not very different. The cow ganglia contained 24% type I ganglionic interneurons, the remaining 76% being classified as type II. Of all the small, fluorescent cells examined in the feline superior cervical ganglion, only 2% were type I; the rest were type II cells. Thus type I cells are common in the cow, whereas they are virtually absent in the cat. As the dopamine receptor-adenylate cyclase complex is located on the postsynaptic membrane of the synapse between the interneurone and the principal ganglionic neurone, the scarcity of ganglionic interneurons (type I small, intensely fluorescent cells) in the cat ganglion may account for the meagre response to dopamine in this species.

These results raise the question: are all small, intensely fluorescent cells interneurons? The answer may well be no. There exist at least two types of small, intensely fluorescent cells, one of which (type I) can be compared with the classical interneurone first described in the rat by Williams<sup>1,2</sup>. The other (type II) is probably not an interneurone and is (at least in the bovine and feline superior cervical ganglion) associated with blood vessels, leading one to suspect a functional association with these vessels.

In summary, there are two main types of small, intensely fluorescent cell in the superior cervical sympathetic ganglion. We have noted a virtual absence of the type I cell (comparable with the interneurone in the rat) in the cat. Also absent or much reduced in the cat is the dopamine-sensitive adenylyl cyclase receptor mechanism involved in neural transmission in other species. We therefore infer that the type I cell is a ganglionic interneurone capable of activating this receptor mechanism. Type II remains an enigma.

T. H. WILLIAMS  
A. C. BLACK, JR  
T. CHIBA  
R. C. BHALLA

Department of Anatomy,  
University of Iowa,  
Iowa City, Iowa 52242

Received February 18; accepted May 28, 1975.

- <sup>1</sup> Williams, T. H., *Nature*, 214, 309–310 (1967).
- <sup>2</sup> Williams, T. H., and Palay, S. L., *Brain Res.*, 15, 17–34 (1969).
- <sup>3</sup> Greengard, P., McAfee, D. A., and Keibarian, J. W., in *Advances in Cyclic Nucleotide Research*, 1 (edit. by Greengard, P., Robison, G. A., and Paoletti, R.), 337–355 (Raven, New York, 1972).
- <sup>4</sup> Greengard, P., Keibarian, J. W., and McAfee, D. A., in *Proc. fifth Int. Congr. Pharmacol., San Francisco 1972*, 5, 207–217 (1973).
- <sup>5</sup> Greengard, P., and Keibarian, J. W., *Fedn Proc.*, 33, 1059–1067 (1974).
- <sup>6</sup> McAfee, D. A., and Greengard, P., *Science*, 178, 310–312 (1972).
- <sup>7</sup> Black, A. C., Jr, Bhalla, R. C., and Williams, T. H., *Progr. Soc. Neurosci., fourth Ann. Meeting, St. Louis, Missouri, Abstract 72* (1974).
- <sup>8</sup> Keibarian, J. W., and Greengard, P., *Science*, 174, 1346–1349 (1971).
- <sup>9</sup> Black, A. C., Jr, Bhalla, R. C., and Williams, T. H., *Anat. Rec.*, 178, 311 (1974).
- <sup>10</sup> Grillo, M. A., Jacobs, L., and Comroe, J. H., Jr, *J. comp. Neurol.*, 153, 1–14 (1974).
- <sup>11</sup> Chiba, T., Black, A. C., Jr, and Williams, T. H., *Anat. Rec.*, 181, 331 (1975).
- <sup>12</sup> Gilman, Alfred G., in *Advances in Cyclic Nucleotide Research*, 2 (edit. by Greengard, P., Robison, G. A., and Paoletti, R.), 9–24 (Raven, New York, 1972).

## pH-sensitive cells at ventro-lateral surface of rat medulla oblongata

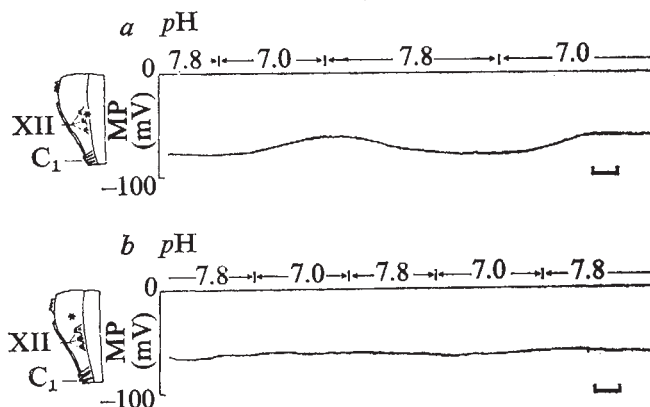
MARKED effects of pH changes in the cerebrospinal fluid (CSF) on ventilation have been confirmed by a number of investigators<sup>1</sup>. Consistent augmentation of respiration by applying acid fluids

to specific regions on the ventro-lateral surface of the cat medulla oblongata<sup>2,3</sup> has been shown, and similar results were observed with the brain stem of the rat<sup>4</sup>. The chemosensitive nature of these regions proposed from the above experiments has been substantiated by electrophysiological studies using an extra-cellular recording technique<sup>5,6</sup>. Movements of the brain as a result of respiration and cardiac activity, however, make it difficult to obtain intracellular recordings of nerve activity in these structures. Therefore, thin brain slices were used *in vitro* in the present experiment.

The brain stem of the rat at the level from the 1st cervical nerve to the roots of the vagal and glossopharyngeal nerves was excised under intraperitoneal chloralose (60 mg kg<sup>-1</sup>)-urethane (250 mg kg<sup>-1</sup>) anaesthesia. A thin brain slice (0.3–0.5 mm thick) was dissected from either side of the ventral surface of the medulla as described previously<sup>7</sup>. Preparations were placed on a nylon mesh in the incubation chamber, ventral surface upwards, and perfused continuously with mock CSF at various pH (7.0–7.8) at 36 °C. Normal mock CSF (pH 7.4) had the following composition (mM): NaCl, 125; NaHCO<sub>3</sub>, 25; KCl, 3.5; CaCl<sub>2</sub>, 1.3; MgCl<sub>2</sub>, 1.1; NaH<sub>2</sub>PO<sub>4</sub>, 0.51; urea, 2.2; and glucose, 3.4. A high or low pH solution was obtained by altering the NaHCO<sub>3</sub> concentration with constant P<sub>CO<sub>2</sub></sub>. Solutions were equilibrated by a gas mixture containing 5.6% CO<sub>2</sub> in oxygen (P<sub>CO<sub>2</sub></sub> 42 mmHg). In another series of experiments the P<sub>CO<sub>2</sub></sub> was varied from 18 to 90 mmHg at constant pH. Transmembrane potentials were recorded using glass micropipettes filled with 3 M KCl having a resistance of 20–30 MΩ.

After 30 min incubation, a microelectrode was inserted into various parts of the surface layer (up to about 200 μm deep) and negative potentials from –50 to –75 mV, which indicated cell membrane activity, could be recorded at the regions lateral to the pyramid. The mean (±s.e.) membrane potential was –62±2 mV at pH 7.4 and no significant differences in the membrane potential were seen in cells located at different portions of the ventral medulla. When the pH of the perfusing solution was altered from 7.0 to 7.8, however, cells located at the area medial to the root of XIIth cranial nerve, just lateral to the pyramid, showed apparent potential changes. Figure 1a shows a continuous recording of membrane potential from a single cell of this area. The membrane potential of this cell was about –78 mV at pH 7.8 and was depolarised gradually to about –58 mV by application of a low pH (7.0) solution. The average membrane potential of cells in this area was –55 and –75 mV at pH 7.0 and 7.8, respectively. We were unable

**Fig. 1** Effect of changes in pH of perfusing solution on membrane potential of cells at the surface layer of the rat ventral medulla *in vitro*. *a*, Record from a single cell at the area medial to the XIIth cranial nerve (Loeschcke's area). Note the significant changes in potential with changes in pH. *b*, Record from a single cell at 1 mm cranial to the root of XIIth cranial nerve and 1 mm lateral to the pyramid. In both *a* and *b* pH was changed with constant P<sub>CO<sub>2</sub></sub> (42 mmHg). MP, Transmembrane potential; XII, XIIth cranial nerve; C<sub>1</sub>, 1st cervical nerve. Asterisk in schematic representation of the ventral medulla represents site at which potential was recorded. Bar, 1 min.



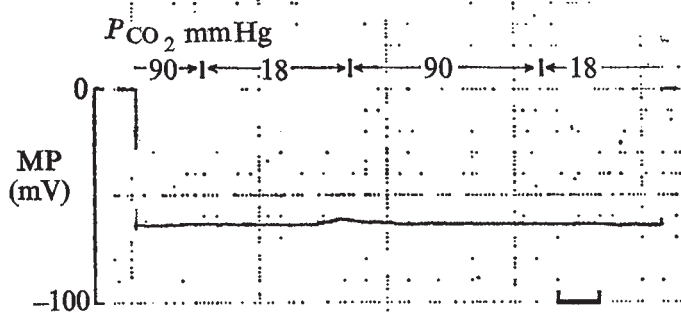


Fig. 2 Effect of changes in  $P_{CO_2}$  of perfusing solution on membrane potential of a cell at the area medial to the root of XIIth cranial nerve *in vitro*. pH was kept constant at 7.4, adjusting  $NaHCO_3$  concentration. MP, Transmembrane potential. Bar, 1 min.

to record apparent spontaneous discharges from these cells during depolarisation when a low pH solution was applied. The membrane potential of cells in other areas did not change with alteration in pH of the perfusing solution, which indicates that cells have no sensitivity to changes in the hydrogen ion concentration of the extracellular fluid (Fig. 1b). On the other hand, when the  $P_{CO_2}$  of the perfusing solution was altered, without altering the pH by adjusting the  $NaHCO_3$  concentration, membrane potentials of cells of all the surface area remained constant.

Figure 2 was obtained from a single cell located at the root of XIIth cranial nerve. In this case the membrane potential was about  $-64$  mV at pH 7.4 with  $P_{CO_2}$  42 mmHg and the potential was maintained as the  $P_{CO_2}$  was changed from 90 to 18 mmHg. Therefore, cells located at the area medial to the root of XIIth cranial nerve (Loeschcke's area) seem to be characterised by being sensitive specifically to extracellular pH and not to  $P_{CO_2}$ . The same characteristics of unique pH sensitivity have also been demonstrated by *in vivo* studies<sup>3,5,6</sup> in which ventilation and neuronal discharges recorded from the ventral medulla were observed with the same perfusion experiments.

The site of localisation of pH-sensitive cells is in accord with the area at which the application of low pH solution causes the highest and most rapid augmentation of respiration in the cat *in vivo*<sup>1,3</sup>. Other areas which have been responsible for the respiratory chemosensitivity to changes in CSF pH have been located at the surface of the ventral medulla near the roots of the vagal and glossopharyngeal nerves (Mitchell's area)<sup>1</sup>. In the present experiment, however, there was no observed change in potentials recorded from the cells of this area with changes in pH or  $P_{CO_2}$ . It is suggested that cells located in deeper (more than 200  $\mu$ m) layers of this area may be sensitive to pH (ref. 8).

pH or  $P_{CO_2}$ -sensitive cells have been reported in the abdominal ganglion of the marine mollusc *Aplysia*<sup>9,10</sup>, and have also been found in cortical and spinal neurones of the rat<sup>11,12</sup>. In the present experiment pH-sensitive cells were shown to exist in the specific region of the medulla which has been assumed to be involved in the chemical regulation of respiration<sup>1</sup>. Whether this cell will exhibit spontaneous activity *in vivo* and be responsible for driving the ventilation or not, is not yet known. It is possible, however, that respiratory responses to changes in the pH of the CSF or brain extracellular fluid, and to changes in acid-base status of blood, may partly be initiated through this cell.

Y. FUKUDA  
Y. HONDA

Department of Physiology,  
Chiba University School of Medicine,  
Chiba, Japan

Received April 21; accepted May 28, 1975.

- <sup>1</sup> Loeschcke, H. H., in *Respiratory Physiology* (edit. by Widdicombe, J. G.), 167 (Butterworths, London, 1974).
- <sup>2</sup> Mitchell, R. A., Loeschcke, H. H., Massion, W. H., and Severinghaus, J. W., *J. appl. Physiol.*, 18, 523 (1963).

- <sup>3</sup> Schläpke, M. E., See, W. R., and Loeschcke, H. H., *Resp. Physiol.*, 10, 198 (1970).
- <sup>4</sup> Hori, T., Roth, G. I., and Yamamoto, W. S., *J. appl. Physiol.*, 28, 721 (1970).
- <sup>5</sup> Schläpke, M. E., See, W. R., Herker, A., Luttmann, A., and Loeschcke, H. H., *Pflügers Arch.*, 347, R38 (1974).
- <sup>6</sup> Schläpke, M. E., See, W. R., and Kille, J. F., *Proc. Int. Union physiol. Sci.* (XXVI), abstract 269 (1974).
- <sup>7</sup> Yamamoto, C., and McIlwain, H., *J. Neurochem.*, 13, 1333 (1966).
- <sup>8</sup> Dermietzel, R., in *Acid-base Homeostasis of Brain Extracellular Fluid* (Abstract of symposium, Bochum, 1975).
- <sup>9</sup> Chalazonitis, N., *Ann. N.Y. Acad. Sci.*, 109, 451 (1963).
- <sup>10</sup> Brown, A. M., and Berman, P. R., *J. gen. Physiol.*, 56, 543 (1970).
- <sup>11</sup> Speckmann, E. J., and Caspers, H., *Pflügers Arch.*, 310, 235 (1969).
- <sup>12</sup> Speckmann, E. J., Caspers, H., and Sokolov, W., *Pflügers Arch.*, 319, 122 (1970).

## BCG suppresses growth and metastasis of hydatid infections

THE asexually proliferating larvae of the cestode *Echinococcus multilocularis* have an intriguing resemblance to malignant tumours. Indeed, until 1856 when Virchow demonstrated the parasitic nature of these cyst masses in man, such lesions were commonly diagnosed as carcinomas of the liver<sup>1</sup>. In the laboratory, infections with this parasite can be propagated vegetatively by transplanting the metacystode into the peritoneal cavity of susceptible rodent intermediate hosts<sup>2</sup>. The protoscolices transform into small cysts which grow and metastasise by peripheral herniation and the detachment of minute vesicles<sup>3</sup>.

Macrophages from donors which have been inoculated with microorganisms such as *Besnoitia*, *Toxoplasma* or BCG can nonspecifically destroy tumour cells *in vitro*<sup>4-6</sup>. BCG has been successfully used in the active, nonspecific immunotherapy of malignant tumours *in vivo*<sup>7,8</sup> and we report here that this same treatment may also be effective in the immunoprophylaxis and immunotherapy of *E. multilocularis* infections.

Pretreatment of the experimental cotton rat host (*Sigmodon hispidus*) with  $26.4 \times 10^6$  colony-forming units (CFU) of BCG suppressed the growth and metastasis of an intraperitoneal inoculum of 200 protoscolices (Table 1). Thus, autopsy 50 d after inoculation revealed that only two of the four BCG-treated cotton rats of this group had developed echinococcosis. These two infected animals bore cyst masses weighing 0.5 g and 1.5 g which were composed of one and two foci respectively. In contrast, the four untreated control animals bore an average hydatid cyst weight of 14 g ( $P < 0.01$ ) composed of an average of 40.5 distinct foci ( $P < 0.005$ ).

BCG treatment 2 weeks after intraperitoneal inoculation of 200 protoscolices did not limit the establishment and growth of the hydatid infection. All animals within this group developed infections the weights of which were indistinguishable from controls ( $P > 0.10$ ). BCG treatment after inoculation did, however, severely suppress the number of parasitic foci of infection which developed from the inoculum. Whereas controls bore an average of 40.5 foci of infection, treated animals bore only 4.8 ( $P < 0.005$ ); the number of foci were also significantly greater ( $P < 0.025$ ) in this group than in that treated before infection. The severity of this suppression is underlined by the fact that one protoscolex can give rise to more than 20 foci of infection by metastasis in cotton rats<sup>9</sup>.

Our study indicates that prior BCG treatment suppresses the growth and metastasis of an intraperitoneal infection with *E. multilocularis*. BCG treatment of established infections, on the other hand, only limits the parasite metastases, whereas growth (assessed by the total weight of the cyst biomass) seems to proceed normally. These findings further emphasise the striking similarities between this parasitic infection and the growth and proliferation of malignant tumours. Baldwin and Pimm<sup>10</sup> have reported that pulmonary metastases from primary rat hepatomata can be similarly restricted by BCG treatment. They report that, again, this treatment had no influence on the occurrence or growth



Table 1 Effect of BCG treatment on growth and metastasis of *E. multilocularis*

|      | Untreated infected controls   |             | BCG treatment 1 week before inoculation |             | BCG treatment 2 weeks after inoculation |             |
|------|-------------------------------|-------------|-----------------------------------------|-------------|-----------------------------------------|-------------|
|      | Total weight of cyst mass (g) | No. of foci | Total weight of cyst mass (g)           | No. of foci | Total weight of cyst mass (g)           | No. of foci |
|      | 10.5                          | 47          | 1.5                                     | 2           | 8.8                                     | 3           |
|      | 9.0                           | 29          | 0.0                                     | 0           | 12.0                                    | 3           |
|      | 12.6                          | 36          | 0.5                                     | 1           | 20.0                                    | 8           |
|      | 23.9                          | 50          | 0.0                                     | 0           | 12.7                                    | 5           |
| Mean | 14.0                          | 40.5        | 0.5                                     | 0.8         | 13.4                                    | 4.8         |

Twelve 5-week-old cotton rats were each inoculated with 200 protoscolices of *E. multilocularis*, and autopsied 50 d later for hydatid cyst masses. Eight of these animals had been treated with an intraperitoneal injection of  $26.4 \times 10^6$  CFU of lyophilised BCG (lot no. 1707-6; Institut de Microbiologie et d'Hygiène de Montréal) in 0.5 ml distilled water. Four of these latter animals had been treated 1 week previously and the other four rats 2 weeks after inoculation with the parasite.

rates of primary tumours (assessed by the survival of experimental animals).

The treatment dose of  $26.4 \times 10^6$  CFU of BCG was tolerated well by our animals although all cotton rats treated before or after inoculation with the parasite developed small intraperitoneal granulomatous lesions.

We are therefore continuing the search for a treatment regimen that will maximise the immunoprophylactic and immunotherapeutic effects of BCG in hydatid disease without the production of granulomas.

This work was supported by a National Health grant. Research at the Institute of Parasitology is also supported by the NRC of Canada.

M. E. RAU  
C. E. TANNER

Institute of Parasitology,  
McGill University, Macdonald College,  
Quebec, Canada H0A 1C0

Received May 12; accepted June 2, 1975.

- Virchow, R., *Verh. Phys.-Med. Ges. Würzburg*, 6, 84-95 (1856).
- Lubinsky, G., *Can. J. Zool.*, 38, 149-151 (1960).
- Webster, G. A., and Cameron, T. W. M., *Can. J. Zool.*, 34, 877-891 (1961).
- Hibbs, J. B., Jr., Lambert, L. H., Jr., and Remington, J. S., *J. Infect. Dis.*, 129, 587-592 (1971).
- Hibbs, J. B., Jr., Lambert, L. H., Jr., and Remington, J. S., *Nature new Biol.*, 235, 48-50 (1972).
- Cleveland, R. P., Meltzer, M. S., and Zbar, B., *J. natn. Cancer Inst.*, 52, 1887-1895 (1974).
- Bluming, A. Z., Vogel, C. L., Ziegler, J. L., Mody, N., and Kamya, G., *Ann. intern. Med.*, 76, 405-411 (1972).
- Gutterman, J. U., Mavligit, G., McBride, C., Frei, E., and Hersh, E. M., *Lancet*, i, 1208-1212 (1973).
- Rau, M. E., and Tanner, C. E., *Can. J. Zool.*, 51, 55-59 (1973).
- Baldwin, R. W., and Pimm, M. V., *Br. J. Cancer*, 30, 473-476 (1974).

## Host growth induced by genetic tumour grafts

SPONTANEOUS tumours in *Nicotiana* were first reported by Kostoff<sup>1</sup> and are called genetic tumours<sup>2</sup>. They arise on certain interspecific tobacco hybrids<sup>2</sup>, and it has been proposed that certain genes when appropriately combined in a hybrid promote the development of tumours<sup>3,4</sup>. Though in bacterially induced crown gall tumours the tumour state is transmissible from a tumour cell to a normal cell in the absence of viable bacteria<sup>5,6</sup>, efforts to demonstrate an ability of genetic tumours to induce tumour development across a graft union have failed<sup>2</sup>. Attempts to graft *Nicotiana glauca* × *Nicotiana langsdorffii* hybrid tumour tissues from sterile culture to non-parental species of *Nicotiana* were unsuccessful because the grafts died<sup>7,8</sup>. Here we report the successful grafting of *N. glauca* × *N. langsdorffii* amphidiploid (GGLL) tissue from sterile culture on to *N. tabacum* cv. Wisconsin 38. Within a month of grafting, the graft (GGLL tumour tissue) induced the development of host (*N. tabacum*) shoots in wound sites and some grafts became chimaeral.

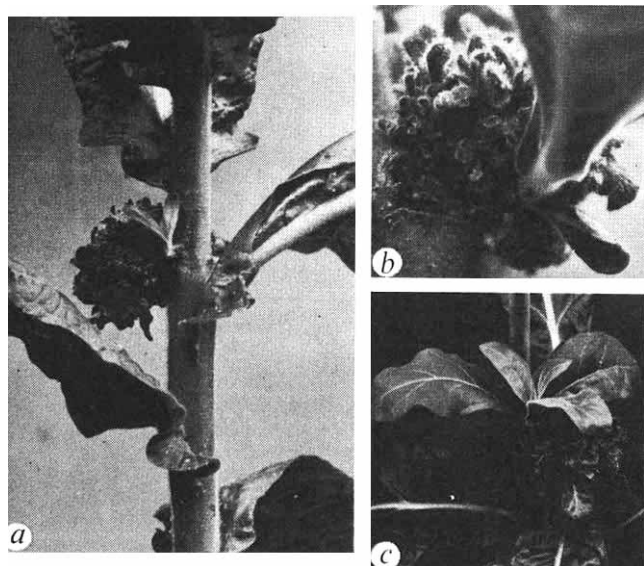
We shall describe one of the three separate grafting experiments we performed. Fifteen grafts were made, 11

produced GGLL tumours, and four grafts induced the development of one or more host shoots within a month of grafting. In one graft the shoot came from the side of the tumour, in another from the tumour region (Fig. 1a), and in the other two cases shoots came from the wound area where the graft did not take. Sussex *et al.*<sup>9</sup> never observed shoot development from comparably wounded internodal areas of the same strain of *N. tabacum*, and we have wounded plants and have grafted numerous pieces of *N. tabacum* tissues into *N. tabacum* plants without ever observing shoot development from an internodal wound.

One shoot growing out of a graft (Fig. 1b) was rooted and the genotype of its tissue layers was determined. Its morphology was *N. tabacum*, its seed germinated into *N. tabacum* plants, and its pith regenerated in culture to produce *N. tabacum* plants. Thus the three layers in its shoot apical meristem, T<sub>1</sub>, T<sub>2</sub>, and C, were genetically *N. tabacum*. In another instance a sectorial chimaera was produced (Fig. 1c). As this shoot grew, the GGLL sector was eliminated, but by removing the apex, we forced an axillary bud from the GGLL sector to develop. This bud produced a shoot which was also a mixture of graft and host cells as indicated by tumour production, leaves of intermediate shape, and flowers with morphological characters of both GGLL and *N. tabacum*.

Bacteria-free crown gall tumour cells are reported to produce or contain one or more substances which cause neighbouring cells to become tumorous<sup>5,6</sup>. As indicated from previous work<sup>2,7,8</sup> and from the results presented here, it is unlikely that genetic tumour cells can influence cells with a non-tumorous genotype to become tumorous. The genetic tumour graft does, however, influence host cells to divide and organise shoot meristems. Since host shoots emerged from graft tissue, the tumours must in some cases be chimaeral, and this assumption is strengthened by the observation of one chimaeral shoot. Although it is possible that tumorous host cells exist among the graft cells, it is more plausible that host cells are induced to divide under the influence of graft growth regulators of the auxin and cytokinin types. That is, GGLL tumours produce auxin and cytokinin<sup>10</sup>, and when *N. tabacum* plants are wounded and the wound exposed to these growth regulators, shoot meristems develop (C.N.M., unpublished). In addition, no growth is observed on the host side of the graft union whereas cell division and growth occur on the host side of the graft union during tumour induction with sterile bacterial tumour tissue<sup>5,6</sup>.

Although crown gall tumours and genetic tumours exhibit similar hormonal autonomy in tissue culture and retention of abnormal growth on grafting<sup>11</sup>, these results suggest there is a fundamental difference in their capacity to influence non-tumorous cells of a host into which they are grafted. Genetic tumours arise spontaneously or appear following injury or external hormone application<sup>2,12</sup>. In the case of crown gall, both injury and the presence of



**Fig. 1** GGLL tumour tissue was grown in sterile culture on Linsmaier and Skoog medium<sup>15</sup> without hormonal supplement. Small pieces (0.075 to 1.0 cm<sup>2</sup>) of GGLL tumour were grafted to the top or into a V-shaped lateral notch in the stem of greenhouse grown *N. tabacum* plants. Before grafting, plants were decapitated, and graft tissue was always placed in an internodal area. Plants were used after bolting but before anthesis (flowering) and axillary buds were usually removed as they grew out. *a*, *b*, *N. tabacum* shoots growing out of grafted tumour tissue; *c*, chimaeral shoot growing out of graft tissue. Note leaf in upper right hand corner: upper half is *N. tabacum* and lower half is GGLL. Magnification: *a*,  $\times 0.38$ ; *b*,  $\times 1.5$ ; *c*,  $\times 0.2$ .

bacteria are required for tumour initiation. Since it has not been possible to demonstrate the presence of foreign DNA in sterile bacterial tumour cells<sup>13,14</sup>, the possible genetic relationship between the suspected 'tumour inducing principle' elaborated by the bacterium and the ability of bacteria-free tumour cells to induce tumour development is unclear. The fact that genetic tumour cells cannot transmit their tumour state though crown gall cells can, suggests that genetic tumour development and maintenance results from endogenous, non-transmissible gene regulation whereas crown gall could be elicited and maintained by a transmissible factor of external origin.

We thank A. DeMaggio, T. Sachs, and K. Walker for their encouragement and comments, and H. H. Smith for GGLL seed. This work was supported by grants from the American Cancer Society; and the National Institutes of Health.

CARL N. MCDANIEL  
IAN M. SUSSEX

Department of Biology,  
Yale University,  
New Haven, Connecticut 06520

## Target cell for oncogenic action of polycythaemia-inducing Friend virus

ONCORNAVIRUSES carry information for two distinct functions: virus multiplication and expression of oncogenicity<sup>1,2</sup>. The polycythaemia-inducing Friend virus (FVP) (as the Rauscher leukemia virus) infects and replicates in many types of cells cultured *in vitro* but as yet, no system for both infection *in vitro* and subsequent expression of oncogenic function has been described<sup>3,4</sup>. Morphological transformation of unidentified target cells occurs *in vivo* after infection of susceptible mice by FVP<sup>5</sup>. Transformation is characterised by the rapid and massive appearance of hyperbasophilic cells, so called Friend cells, only in the spleen red pulp, about 30–40 h after virus inoculation. Thereafter, an erythrocytic maturation starts and proceeds without influence of endogenous or exogenous erythropoietin, the physiological humoral factor for normal erythropoietic differentiation. The number of hyperbasophilic Friend cells and erythroblasts increases; these cells invade the organism and the animal dies 1–2 months after infection.

Oncogenic potencies *in vivo* could result from the need for a precise cell type or cell function to enable the expression of the oncogene<sup>6</sup>. Haematopoietic cells are required for the initiation of Friend disease<sup>7</sup>. Different authors have concluded that the target cells (that is those cells which become hyperbasophilic and multiply after virus infection) for viral oncogenic action *in vivo* were, variously, the multipotent stem cells of the haematopoietic system<sup>8</sup>, or a myelopoietic cell<sup>9</sup>. Others claimed that the target cells were probably the erythropoietin responsive cells or closely related precursors<sup>5,6</sup>.

From these indirect results, the answers to at least three questions remain unclear. First, is the multipotent stem cell of the haematopoietic system, the so-called colony forming unit, a target cell for oncogenic action of the virus? Second, is the target cell a precursor of the erythrocytic line only? Third, do the transformed hyperbasophilic cells differentiate only along the erythrocytic line?

Here we present results which, in our opinion, give direct answers to these questions, by using a method which could be easily adapted for other leukaemia viruses.

We took advantage of the well known property of the haematopoietic stem cells; they give rise, in the spleen of irradiated syngeneic recipients, to macroscopic colonies constituted of differentiated haematopoietic cells<sup>10</sup>. Nine days after the graft, most of the colonies contain only a single haematopoietic cell line either erythroid, neutrophilic granuloid, megakaryocytic or eosinophilic granuloid in a decreasing order of frequency<sup>11</sup>. Polycythaemia induced by intraperitoneal administration of packed syngeneic red cells into mice stopped the production of endogenous erythropoietin and resulted in the suppression of the erythroid spleen colonies<sup>12</sup>. Such suppressed erythroid colonies remain in the spleen as small undifferentiated colonies and do not convert to another cell type<sup>13</sup>. Three or four days after the cell graft, erythropoietin responsive cells appear in these small colonies since administration of exogenous erythropoietin results in the immediate appearance of pronormoblasts. As postulated by Lajtha, this capacity to respond to erythropoietin is achieved only after a number of cell cycles<sup>14</sup> (Fig. 1).

The experimental data were the following: hypertransfused, irradiated C3H/Hemg mice (Carshalton, England) were grafted with  $3 \times 10^5$  syngeneic normal spleen cells (this number of cells give rise to a mean number of 10 colonies per spleen). On days 2, 4 or 6 after the graft, the mice were inoculated intravenously with 0.2 ml of highly infectious plasma ( $10^5$  focus forming units (FFU)) from polycythaemic

Received April 28; accepted May 22, 1975.

- <sup>1</sup> Kostoff, D., *Zbl. Bakt. Parasit. de*, II abt., **81**, 244–260 (1930).
- <sup>2</sup> Smith, H. H., *Progr. exp. Tumour Res.*, **15**, 138–164 (1972).
- <sup>3</sup> Ahuja, M. R., *Molec. gen. Gent.*, **103**, 176–184 (1968).
- <sup>4</sup> Näf, U., *Growth*, **22**, 167–180 (1958).
- <sup>5</sup> Aaron-Da Cunha, M. I., *C. r. heb. Séanc. Acad. Sci., Paris*, **268**, 318–321 (1969).
- <sup>6</sup> Meins, Jr., F., *Differentiation*, **1**, 21–25 (1973).
- <sup>7</sup> White, P. R., *Cancer Res.*, **4**, 791–794 (1944).
- <sup>8</sup> White, P. R., and Braun, A. C., *Cancer Res.*, **2**, 597–617 (1942).
- <sup>9</sup> Sussex, I. M., Clutter, M. E., and Goldsmith, M. H. M., *Am. J. Bot.*, **59**, 797–804 (1972).
- <sup>10</sup> Schaeffer, G. W., and Smith, H. H., *Pl. Physiol.*, **38**, 291–297 (1963).
- <sup>11</sup> Braun, A. G. (ed.), *Progr. exp. Tumour Res.*, **15** (1972).
- <sup>12</sup> Schaeffer, G. W., *Nature*, **196**, 1326–1327 (1962).
- <sup>13</sup> Chilton, M., et al., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3672–3676 (1974).
- <sup>14</sup> Drlica, K. A., and Kado, C. I., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3677–3681 (1974).
- <sup>15</sup> Linsmaier, E. M., and Skoog, F., *Physiol. Pl.*, **18**, 100–127 (1965).



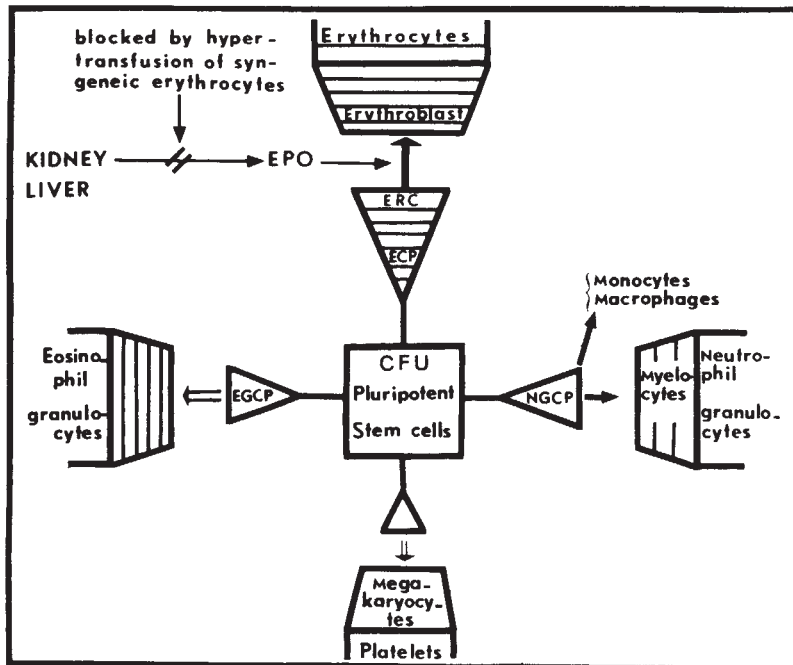


Fig. 1 Simplified schema of the murine haematopoietic system. Normal steady state<sup>14</sup>: The pluripotent haematopoietic stem cell compartment (CFU) is a self-maintaining population. A 'first step' differentiation event transforms the pluripotent stem cells into committed precursor cells (ECP, erythroid committed precursor; NGCP, neutrophilic granuloid committed precursor; EGCP, eosinophilic granuloid committed cells). These transit populations undergo a number of cell cycles during their maturation but without the capability for prolonged self maintenance. A 'second step' differentiation triggers the late forms of the committed cells and initiates the maturation process of the identifiable haematopoietic cell line. The mechanisms or factors which control the different haematopoietic cell line productions are as yet ill defined. The 'second step' differentiation is well understood in the case of erythropoiesis. A humoral factor erythropoietin (EPO) has been demonstrated to stimulate erythropoietin responsive cell (ERC) proliferation and recruitment, to induce ERC differentiation and to promote the erythropoietic maturation. Hypertransfused-plethoric state: Injections of washed syngeneic red blood cells suppress EPO production and result in the arrest of the erythroid maturation process. Identifiable erythroid cells disappear from haematopoietic organs but ERC remain present at any time. There is abundant evidence that the ECP is constantly supplied by an outflux from the pluripotent compartment and an intrinsic rate of ERC self-replication<sup>15</sup>.

mice infected with FVP. Three days after virus inoculation, the spleens were removed, fixed in Helly's solution or buffered formalin with added saccharose, embedded in paraffin or methacrylate and sliced sagittally into 2–3  $\mu$ m sections. Sections were stained with diorthoanisidine, an indicator of the peroxidase activity of haemoglobin, and with azur B and fuchsin acid for detection of basophilic and acidophilic cells. Haemoglobin synthesis was also investigated by autoradiographic determination of <sup>59</sup>Fe incorporation. <sup>59</sup>Fe (45  $\mu$ Ci per mouse, specific activity 3.85 Ci g<sup>-1</sup>) was injected intravenously as ferric chloride, 3 h before killing. Spleen sections mounted on glass slides were dipped in Ilford K5 emulsion and exposed for 3–4 weeks.

Spleens of mice injected with FVP on d 4 after grafting and harvested on d 7, contained as expected, the differentiated neutrophilic, megakaryocytic and eosinophilic colonies. In addition, there were small colonies of hyperbasophilic Friend cells (20–50 per section) in which baso-

philic and some polychromatophilic erythroblasts, positive to diorthoanisidine staining, were observed. The labelling of both typical hyperbasophilic Friend cells and erythroblasts in autoradiograms confirmed initiation of haemoglobin synthesis.

Spleens of mice killed 9 d after cell graft, that is 3 d after infection of FVP (day +6), contained similar but larger colonies. In these mice as well as in the former group, the distribution of the various histological types of colonies (erythroid 52%; granulocytic 28%; megakaryocytic 9%; mixed 5%; and undifferentiated 6%) was not significantly different from the distribution of colonies in spleens of non-plethoric-irradiated mice receiving the same spleen cell suspension. This indicates that practically all the presumptive erythroid colonies contained target cells for the virus. In the 9-d-old erythroid colonies obtained after injection of spleen cells into plethoric-irradiated and virus-infected hosts, the ratio of maturing erythroblasts to the large

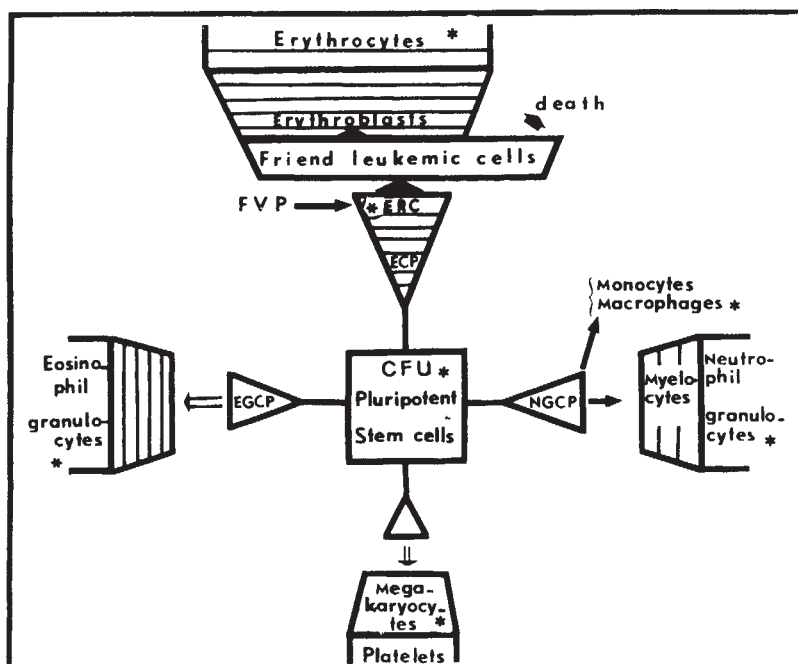


Fig. 2 Schema of the leukaemogenesis by Friend leukaemia virus. Oncogenic potency of the Friend virus (FVP) is expressed only at the 'late' erythroid committed precursor level. After viral transformation Friend leukaemic cells multiply and differentiate along the erythrocytic pathway. EPO is not required to initiate the pathological erythropoietic differentiation. As indicated by kinetic studies, Friend leukaemic cells are not self-maintaining<sup>16</sup> (they die or differentiate). Then the progression of the disease requires a constant recruitment, of target cells from the ECP and CFU compartments. The viral replicative ability is not restricted to the erythroblastic cells.

\*The most primitive haematopoietic stem cells and the different haematopoietic cell lines are infected by the virus but their development remains practically normal.

hyperbasophilic precursor cells was smaller than in the erythroid colonies observed the spleen of irradiated normal mice, suggesting a delayed or ineffective erythropoiesis. Spleens of mice inoculated by the virus on day 2 and killed on day 5 after the cell graft, contained small clusters of 5–10 hyperbasophilic Friend cells in association with only young basophilic erythroblasts. Twenty-four hours earlier (2 d after infection) no hyperbasophilic Friend cells could be observed. Since transformation requires 30–40 h (ref. 5), it can be postulated that the target cells for the virus were present only on or after day 3 or 4. Referring to the age structure of maturation process in the erythroid committed compartment<sup>14</sup>, this suggests that FVP needs a mature precursor cell to express its oncogenic potency.

With respect to the three initial questions, these results directly indicate that: (1) the multipotent stem cell of the haematopoietic system is not a target cell for the virus. Colony forming units (CFUs) are present in all types of colonies, and even, as shown by Trentin *et al.*<sup>11</sup>, in a greater number in the granulocytic colonies. Hyperbasophilic Friend cells were, however, never observed within granuloid nor megakaryocytic colonies. (2) The viral gene which induces transformation can be expressed only in an erythroid committed cell (the erythropoietin responsive cell or a closely related precursor) and transformation is immediately followed by erythroid maturation. (3) Only the erythroid pathway of differentiation is open to hyperbasophilic Friend cells (Fig. 2).

Through use of colony formation by the multipotent haematopoietic stem cell, we have been able to examine further the early interaction between a leukaemia virus and the haematopoietic cells.

We thank Martin Charon and Paule Bucau-Varlet for technical assistance. This study was supported by INSERM.

P. E. TAMBOURIN  
F. WENDLING

Unité de Physiologie Cellulaire de l'INSERM,  
(Institut du Radium) Bâtiment 110,  
91405 Orsay, France

Received February 13; accepted June 2, 1975.

- <sup>1</sup> Huebner, R. J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1087–1094 (1969).
- <sup>2</sup> Martin, G. S., *Nature*, **227**, 1021–1023 (1970).
- <sup>3</sup> Yoshikura, H., Hirokawa, Y., Yamada, M., and Sugano, H., *Jap. J. med. Sci. Biol.*, **20**, 225–236 (1967).
- <sup>4</sup> Wright, B. S., and Lasfargues, J. C., *J. natn. Cancer Inst.*, **35**, 319–327 (1965).
- <sup>5</sup> Tambourin, P., and Wendling, F., *Nature new Biol.*, **234**, 230–233 (1971).
- <sup>6</sup> Brommer, E. J. P., and Bentvelzen, P., in *Unifying Concepts of Leukemia*, 929–934 (S. Karger, Basel, 1973).
- <sup>7</sup> Chirigos, M. A., and Marsh, R. W., *Antimicrob. Agents Chemoth.*, **6**, 489–494 (1966).
- <sup>8</sup> Seidel, H. J., in *Unifying Concepts of Leukemia*, 935–942 (S. Karger, Basel, 1973).
- <sup>9</sup> Thomson, S., *Proc. Soc. exp. Biol. Med.*, **130**, 227–231 (1969).
- <sup>10</sup> Till, J. E., and McCulloch, E. A., *Radiat. Res.*, **14**, 213–222 (1961).
- <sup>11</sup> Trentin, J. J., *Regulation of Hematopoiesis*, 159–186 (Appleton-Century-Crofts, New York, 1970).
- <sup>12</sup> Schooley, J. C., *J. cell. Physiol.*, **68**, 249–262 (1966).
- <sup>13</sup> Curry, J. L., Trentin, J. J., and Wolf, N., *J. exp. Med.*, **125**, 703–720 (1967).
- <sup>14</sup> Lajtha, L. G., and Schofield, R., *Differentiation*, **2**, 313–320 (1974).
- <sup>15</sup> Reissmann, K. R., and Udupa, K. B., *Cell Tissue Kinet.*, **5**, 481–489 (1972).
- <sup>16</sup> Smadja-Joffe, F., Jasmin, C., Malaise, E. P., and Bournoutian, C., *Int. J. Cancer*, **11**, 300–313 (1973).

## Characterisation of human cells transformed *in vitro* by urethane

CELLS derived from several animal species, including mouse<sup>1,2</sup>, hamster<sup>3</sup> and rat<sup>4</sup>, have been transformed *in vitro* from the normal to the malignant state by diverse chemical carcinogens. In similar conditions, however, attempts to transform human cells have usually been unsuccessful and as far as we know, such transformation has been reported only once<sup>5</sup>. This was a case of two cell lines treated with urethane, obtained from siblings with von Recklinghausen's disease, a familial disorder transmitted by an autosomal dominant gene, and characterised by multiple fibromas with a high predisposition to malignant

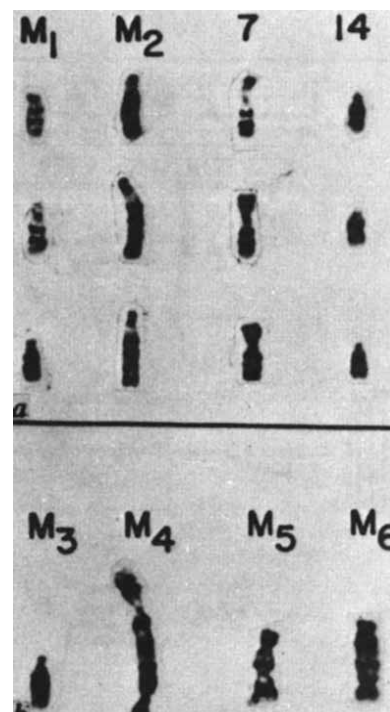
transformation *in vivo*<sup>6</sup>. Although morphologically altered foci of transformed cells were reported, there was the possibility that a few tumour cells in the original population had been selected for by urethane. Therefore we characterised in detail the urethane-treated and untreated cultures. We have found that human cells can indeed be chemically transformed *in vitro*.

The control cells we used were designated as NFS-1. One transformed colony (designated urethane) was derived from a single morphologically altered focus in an NFS-1 culture treated with  $1.1 \times 10^{-2}$  M urethane. A second transformed colony (designated urethane/FeLV) was derived from a single focus obtained in a urethane-treated NFS-1 culture preinfected with feline leukaemia virus. Since morphologically altered foci occurred in numerous urethane-treated cultures<sup>3</sup>, whether or not infected with FeLV, there was no evidence that FeLV was acting as a cocarcinogen. We used this culture for characterisation because it is one of only two continuous cell lines obtained from various attempts to isolate and grow morphologically altered foci.

Trypsin–Giemsa banding<sup>7</sup> revealed (Table 1) no abnormal metaphases in the untreated NFS-1 cells, while all metaphases in the urethane cell line at passage 30 were aneuploid. Most metaphase cells contained 94 or 95 chromosomes. An abnormal marker chromosome ( $M_1$ ) was found in 19 of the 20 metaphases analysed (Fig. 1). An additional marker chromosome ( $M_2$ ) was found in 14 of 20 metaphases (Fig. 1). Thus the transformed cells were derived from a single parent transformed stem cell. Similar marker chromosomes were found at passage 259 (Table 1).

The urethane/FeLV line also had an aneuploid chromosomal pattern at passage 25. This pattern was different from that of the urethane cell line, since most metaphases contained between 68 and 71 chromosomes, and no metaphases had the marker  $M_1$  or  $M_2$  chromosome. But, four additional marker chromosomes,  $M_3$  to  $M_6$ , not found in

Fig. 1 Marker chromosome patterns revealed by the Giemsa–trypsin banding technique: *a*, Similar marker chromosomes,  $M_1$  and  $M_2$ , from the urethane cell line. The normal No. 7 and No. 14 human chromosomes from the same three metaphases are shown for comparison.  $M_1$  probably arose from a deletion of the short arm of a number 7 chromosome. *b*, Representative examples of the marker chromosomes  $M_3$ – $M_6$  found in the FeLV/urethane cell line.  $M_4$ ,  $M_5$  and  $M_6$  are dicentric chromosomes.





**Table 1** Chromosome number and marker chromosome distribution of NFS-1 and urethane or urethane/FeLV transformed cells

| Cell line     | Passage no. | Chromosome no. |    |       |       |       |       |        | Number of metaphases with given markers |                |                |                |                |                |
|---------------|-------------|----------------|----|-------|-------|-------|-------|--------|-----------------------------------------|----------------|----------------|----------------|----------------|----------------|
|               |             | 44-45          | 46 | 65-67 | 68-71 | 72-93 | 94-95 | 96-105 | M <sub>1</sub>                          | M <sub>2</sub> | M <sub>3</sub> | M <sub>4</sub> | M <sub>5</sub> | M <sub>6</sub> |
| NFS-1         | 15          | 3              | 47 |       |       |       |       |        | 0                                       | 0              | 0              | 0              | 0              | 0              |
| Urethane      | 30          |                |    |       |       | 3     | 14    | 3      | 19                                      | 14             | 0              | 0              | 0              | 0              |
|               | 259         |                |    |       |       | 4     | 2     | 14     | 18                                      | 8              | 0              | 0              | 0              | 0              |
| Urethane/FeLV | 25          |                |    | 6     | 14    |       |       |        | 0                                       | 0              | 16             | 7              | 8              | 5              |

the urethane cell line were seen at various frequencies (Fig. 1). The marker chromosome M<sub>3</sub> was found in 16 of 20 metaphases and additional marker chromosomes M<sub>4</sub>, M<sub>5</sub>, and M<sub>6</sub> were found in 7, 8 and 5 of 20 metaphases, respectively (Table 1). This suggests clonal evolution and separate parental origin for both transformed cell lines.

Untreated NFS-1 cells formed no colonies on soft agar, whereas the established urethane cell line (Table 2) formed numerous large colonies.

We also examined the intracellular and extracellular fibrinolytic activity of the NFS-1 cells and the two urethane-treated lines because greater fibrinolytic activity is usually associated with human malignant cells than with their normal counterparts<sup>8</sup>. The control NFS-1 cells contained little fibrinolytic activity (that is, the level found in normal human fibroblast cultures), whereas the transformed cells showed marked fibrinolytic activity (Table 2).

We also tested the urethane cell line for its ability to produce tumours in newborn hamsters treated with antithymocyte serum<sup>8</sup>. Animals injected with  $2 \times 10^6$  control NFS-1 cells were treated with antithymocyte twice weekly for the first 2 months. No tumours developed in 5 months. A similar number of cells from the urethane line, however, produced rapidly growing fibrosarcomas within 3 weeks (Table 2). When placed back in cell culture, they had the same chromosome patterns as the injected human cells, and also showed high fibrinolytic activity (data not shown). The cell line containing FeLV was not tested for tumorigenicity, since we did not wish to risk contaminating our animal facilities with FeLV.

We believe that the cultures described represent the first documented chemical malignant transformation of human cells. The possibility that a few malignant cells were selected from the NFS-1 culture can be excluded for several reasons. First, repeated attempts to develop continuous cell lines from the untreated parent cultures have failed. Second, as previously reported there was no measurable cytotoxicity nor lag in growth in the NFS-1 cells after

treatment with the doses of urethane used<sup>8</sup>. Third, there was no opportunity for contamination of these transformed cell lines with other human cell lines, since no long term human cell line was present in the laboratory where the work was carried out.

The strongest evidence that the two established cell lines were derived from different parent NFS-1 cells is that each line had not only a markedly different model chromosome number but also dissimilar marker chromosomes. Although the original NFS-1 cells could have two distinct malignant parent lines present before treatment with urethane, this is unlikely since previous studies have shown that human malignant tumours are most often derived from one malignant stem cell<sup>9,10</sup>.

It is interesting that both transformed cell lines had high extracellular and intracellular fibrinolytic activity, whereas the parent cell line did not. This is consistent with studies in other species in which the parent cells contained low fibrinolytic activity, whereas chemically transformed cells had a high activity<sup>8</sup>. This marked difference between normal and transformed human cells may be an important criterion for the presence of human transformed cells in attempts to establish a human model system for chemical transformation. We believe that documentation of the malignant nature of these cells in culture indicates that each of the original morphologically altered colonies<sup>8</sup> was also malignant, but could not be developed into continuous cell lines for unknown reasons. This would be consistent with the difficulty in establishing continuous cell lines from human tumour material<sup>11,12</sup>. We therefore conclude that chemical transformation of human cells does occur and is frequently expressed as foci or morphologically altered cells. Tissue culture conditions, however, are usually inadequate for the establishment of long term cell lines from these transformed cells.

This work was supported by a grant from a US Public Health Service and a contract from the National Cancer Institute. W.F.B. is a recipient of a career development

**Table 2** Fibrinolytic activity, growth in soft agar and tumour formation of transformed and untransformed human cells

| Cell line     | Passage no. | Fibrinolytic activity |                  |                  | Colonies in soft agar per $10^4$ cells plated | No. hamsters with tumours<br>No. hamsters injected |
|---------------|-------------|-----------------------|------------------|------------------|-----------------------------------------------|----------------------------------------------------|
|               |             | Extracellular DS      | Intracellular HS | U per mg protein |                                               |                                                    |
| NFS-1         | 15          | 1                     | 1                | 1                | 0                                             | 0/7                                                |
| Urethane      | 25          | 52                    | 76               | 57.1             | 250                                           | 2/3                                                |
|               | 259         | 58                    | 77               | 69.5             | 342                                           | 5/7                                                |
| Urethane/FeLV | 25          | 64                    | 68               | 29.9             | N.D.                                          | N.D.                                               |

Extracellular fibrinolytic activity was measured by plating  $5 \times 10^5$  cells on to  $^{125}$ I-fibrin dishes in medium containing 10% (v/v) corresponding serum (DS, dog serum; HS, human serum).

Fibrinolytic activity after 24 h, is expressed as the percentage of radioactivity released into the supernatant compared with the total radioactivity of  $^{125}$ I-fibrin per dish<sup>8</sup>. Intracellular fibrinolytic activity was assayed in aliquots of cell lysates prepared in Tris-HCl buffer, pH 8.1 (0.1M) containing Triton X-100 (0.5%). The cell lysates (10  $\mu$ l) were incubated for 2 h in 35 mm  $^{125}$ I-fibrin dishes containing 2.5 ml of 1% HS in Tris-HCl, pH 8.1, and the radioactivity released was measured. The radioactivity released by 100 U of urokinase in identical conditions was taken as 100% fibrinolytic activity. One unit of activity was defined as that amount of activity which released 10% of the urokinase activity in 2 h at 37 °C. The protein content in the cell lysates was determined by the method of Lowry<sup>11</sup>. The methods used for the soft agar and tumorigenicity studies were similar to those previously described<sup>8</sup>.

award from the National Institutes of Health. We thank Dr Hart Isaacs for diagnosing the tumours.

WILLIAM F. BENEDICT\*  
PETER A. JONES  
WALTER E. LAUG

Division of Hematology-Oncology,  
Department of Medicine,  
Children's Hospital of Los Angeles,  
and University of Southern California,  
School of Medicine,  
Los Angeles, California 90027

HOWARD J. IGEL  
AARON E. FREEMAN

The Children's Hospital,  
Buchtel Avenue and Bowery Street,  
Akron, Ohio 44308

Received March 12; accepted May 15, 1975.

\*To whom reprint requests should be addressed.

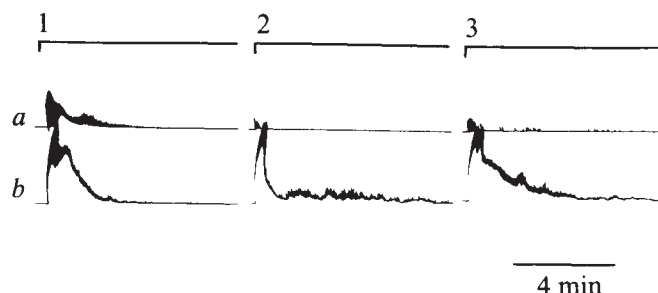
- 1 Chen, T. T., and Heidelberger, C., *Int. J. Cancer*, **4**, 166-178 (1969).
- 2 Reznikoff, C. A., Brankow, D. W., and Heidelberger, C., *Cancer Res.*, **33**, 3231-3238 (1973).
- 3 Berward, Y., and Sachs, L., *J. natn. Cancer Inst.*, **35**, 641-661 (1965).
- 4 Freeman, A. E., Gilden, R. V., Vernon, M. L., Wolford, R. G., Hugunin, P., and Huebner, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2415-2419 (1973).
- 5 Igel, H. J., Freeman, A. E., Spiewak, J. E., and Kleinfeld, K. L., *In Vitro* (in the press).
- 6 Crowe, F. W., Schull, W. J., and Neel, J. V., *A Clinical Pathology and Genetic Study of Multiple Neurofibromatosis* (C. C. Thomas, Springfield, 1956).
- 7 Paul, B., Porter, I. H., and Benedict, W. F., *Humangenetik*, **18**, 185-187 (1973).
- 8 Laug, W. E., Jones, P. A., and Benedict, W. F., *J. natn. Cancer Inst.*, **54**, 173-179 (1975).
- 9 Atkin, N. B., and Baker, M. C., *J. natn. Cancer Inst.*, **36**, 539-557 (1966).
- 10 Porter, I. H., Benedict, W. F., Brown, C. D., and Paul, B., *Expl molec. Path.*, **11**, 340-367 (1970).
- 11 Freeman, A. E., and Price, P. J., in *Proc. Conf. Carcinogenesis Testing of New Drugs* (edit. by Goldberg, L.), 23-25 (Chemical Rubber Company, 1974).
- 12 McAllister, R. M., et al., *Cancer* (in the press).

## Incorporation of acetylcholinesterase into synaptic vesicles is associated with blockade of synaptic transmission

THE transmission of nerve signals across cholinergic synapses involves the presynaptic release (or secretion) of acetylcholine (ACh) which, by acting on specific sites of the postsynaptic membrane, induces changes in the postsynaptic transmembrane potential. According to the vesicle hypothesis<sup>1</sup>, ACh is packed inside synaptic vesicles which release their contents from the nerve terminal on contact with the presynaptic membrane. Although there is abundant experimental evidence supporting the hypothesis<sup>2</sup>, some papers question its validity<sup>3-6</sup>. The most important alternative hypothesis postulates that the releasable ACh is free in the cytoplasm<sup>3-6</sup> and that the vesicles are a secondary reservoir containing 'bound' ACh.

If the vesicle hypothesis is correct, enzymatic destruction of the ACh contained inside the vesicles should inhibit synaptic transmission. A direct way of testing the vesicle hypothesis would be to incorporate into the synaptic vesicles an enzyme that destroys the transmitter substance, and then to measure the resulting effects on transmission. Several groups<sup>7-9</sup> have succeeded in incorporating the enzyme horseradish peroxidase into the synaptic vesicles of various nerve terminals. In some cases<sup>7,9</sup>, this was accomplished by stimulating the nerve terminals at a high rate for enough time to cause vesicle depletion, and then allowing the terminals to rest with the enzyme present in the bathing solution. During the rest period, vesicles containing the enzyme appeared at the terminal. Using this technique, we show here that the incorporation of exogenous acetylcholinesterase (AChE) into the synaptic vesicles of nerve terminals of the frog sartorius neuromuscular junction is associated with a marked inhibition of synaptic transmission, as would be predicted by the vesicle hypothesis.

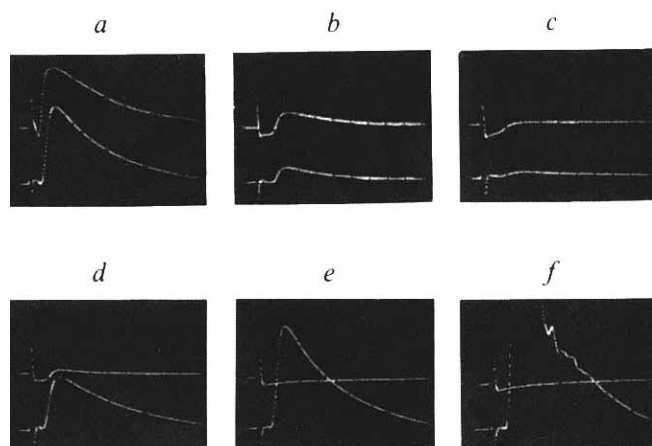
In 16 experiments, non-curarised pairs of sartorius neuromuscular preparations, each pair taken from the same frog,



**Fig. 1** Muscle contractions recorded with force transducers. *a*, Experimental; *b*, control. At the start of period 1, both preparations were stimulated simultaneously at 12 Hz. Stimulation was continued for 20 min in normal Ringer's. At the end of this period, the Ringer's solutions were exchanged for AChE-Ringer's (*a*) or for normal Ringer's (*b*), and the preparations were allowed to rest in the absence of stimulation for 45 min. Shortly before the start of period 2, both solutions were exchanged for normal Ringer's. At 2, a second cycle of stimulation identical to the first was begun. The response of *E* during this period was greatly diminished. Shortly before the end of period 2, the solutions were again exchanged for normal Ringer's, and the preparations were allowed to rest for an additional 45 min. At 3, a third period of stimulation was begun. Little further recovery of *a* was seen during this period.

were simultaneously stimulated through the nerves at 12 Hz for 20 min. At the end of the stimulation, the Ringer's solution bathing the experimental muscle of each pair was exchanged for Ringer's containing 0.5-1.0 mg ml<sup>-1</sup> AChE (Sigma, type V). The control muscle of each pair received either normal Ringer's, Ringer's containing 0.5-1.0 mg ml<sup>-1</sup> bovine serum albumin (Sigma), or Ringer's with paraoxon-inactivated AChE (0.5-1.0 mg ml<sup>-1</sup>) dialysed against Ringer's before use. After a rest of 45 min, the bathing solutions of both experimental and control muscles were exchanged for normal Ringer's. Then the preparations were tested again for muscle contraction in response to nerve stimulation. A typical result is shown in Fig. 1. In most

**Fig. 2** Endplate potentials recorded extracellularly. *a*, Experimental (top trace) and control (bottom trace) were stimulated in normal Ringer's at 20 Hz. Responses were recorded at time 0 (*a*), after 5 min of stimulation (*b*), and after 13 min of stimulation (*c*). After a total of 20 min of stimulation the bathing solutions were exchanged for AChE-Ringer's (experimental, 0.5 mg ml<sup>-1</sup>) or for paraoxon-inactivated AChE-Ringer's (control, 0.5 mg ml<sup>-1</sup>). The preparations then rested for 50 min. Shortly before *d*, the bathing solutions were exchanged for normal Ringer's. The response in *d* was recorded after 50 min of rest, and in *e*, after an additional 45 min of rest. The response in *f* was recorded after 9 min exposure to neostigmine methyl sulphate (0.5 mg ml<sup>-1</sup>). The experimental showed no recovery of endplate potential during the later periods of stimulation, *d*, *e*, or *f*.



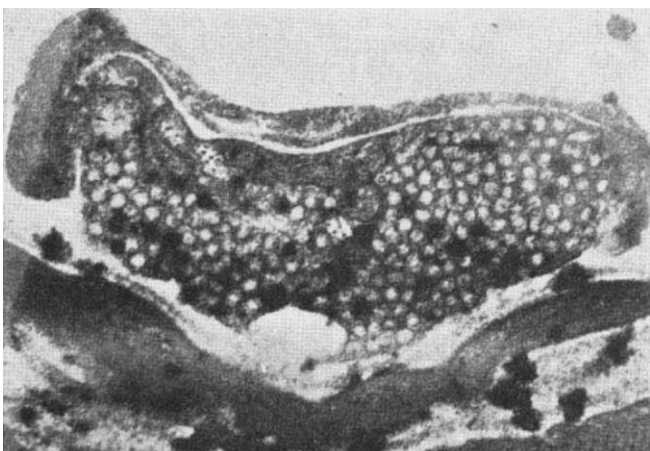
0.2 mV  
10 ms



cases (13 out of 16) the experimental preparation showed an obvious diminution in contraction, whereas the control showed a significant recovery. Occasionally, it was necessary to repeat the cycle of stimulation and rest (in one case, four times) to elicit the effect. The fact that paraoxon-inactivated esterase and albumin did not inhibit transmission indicates that ACh hydrolysis was necessary.

In a different series of experiments, Fatt's method of extracellular recording of endplate potentials (e.p.s) was used<sup>10</sup>. Paired preparations were curarised ( $1\text{--}1.5\text{ mg l}^{-1}$  D-tubocurarine, Abbot) and AChE- and albumin-containing Ringer's were used following the sequence described in Fig. 2. As in non-curarised preparations, the AChE treatment (in seven of nine cases) greatly diminished or abolished the postsynaptic response to nerve stimulation, whereas the response of control preparations recovered to the pre-stimulation value. In these experiments too, the procedure in some cases had to be repeated more than once to obtain the effect. Preincubation of the preparations at  $4^\circ\text{C}$  or brief stimulation during the rest period seemed to interfere with the effect.

Several attempts to reverse the AChE effect were made with both curarised and non-curarised preparations by introducing a second stimulation period (20 Hz, 20 min), in AChE-free Ringer's. Heuser and Reese<sup>7</sup> reported that a second stimulation of terminals already loaded with exogenous horseradish peroxidase resulted in the disappearance of enzyme-containing vesicles. In our experiments, however, no recovery of the AChE-inhibited preparations was observed.



**Fig. 3** Electron micrograph of nerve terminal stained for incorporated AChE ( $\times 67,500$ ). The nerve-muscle preparation was stimulated at 20 Hz for 20 min and rested for 45 min in AChE-Ringer's. After washing several times with normal Ringer's, the preparation was fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate, pH 7.4, and then stained for cholinesterase activity<sup>14</sup>, osmicated, dehydrated, and embedded in Epon. Dimethyl sulphoxide (0.1%) was included in the staining reagent. Black deposits in the synaptic vesicles indicate the presence of AChE activity. Controls which were stimulated and rested in normal Ringer's and then treated as above, had no reaction product in the vesicles. Deposits of reaction product in the synaptic cleft were also seen in controls.

The above mentioned inhibition of the postsynaptic response could be explained by hydrolysis of ACh in the synaptic cleft. There is, in fact, some evidence that AChE can enter and leave the cleft<sup>11-13</sup>. To test this possibility, preparations were stimulated at a slow rate (about  $2\text{ min}^{-1}$ ) in the presence of AChE ( $0.5\text{--}1.0\text{ mg ml}^{-1}$ ) in the Ringer's. With this non-fatiguing stimulation no effects on muscle contraction were observed for several hours. Presumably, vesicle recycling and incorporation of exogenous AChE at this rate of stimulation occurs only very slowly. Additional evidence against the extracellular action of exogenous

AChE is the fact that in preparations where synaptic transmission was completely blocked as described above, the addition of a cholinesterase inhibitor (neostigmine methyl sulphate, K & K,  $1\text{ mg l}^{-1}$ ) did not restore the e.p.s (Fig. 2f).

To determine the location of the exogenous AChE in the presynaptic terminal after stimulation to fatigue and rest in the presence of the enzyme, preparations so treated were fixed, and then stained for the demonstration of cholinesterases according to Crevier and Belanger<sup>14</sup>. In the preparation shown in Fig. 3, 15-20% of the synaptic vesicles contain electron-dense reaction product indicative of cholinesterase activity.

These results show that the incorporation of exogenous AChE into synaptic vesicles is associated with inhibition of synaptic transmission at the frog sartorius neuromuscular junction, suggesting that the ACh available for release is contained in the synaptic vesicles. These results, however, do not rule out the possibility that there is a releasable cytoplasmic store of ACh which is in equilibrium with the intravesicular pool. This latter possibility is compatible with the results of Tauc *et al.*<sup>6</sup> who showed that injection of AChE into the axoplasm of an *Aplysia* cholinergic neurone resulted in inhibition of transmission.

An interesting further application of the technique described here might be the use of this or other transmitter-inactivating enzymes to identify the transmitter substance in different synapses.

This work was supported by the US Public Health Service.

ALBERTO POLITOFF  
ALAN L. BLITZ

Department of Physiology,  
Boston University School of Medicine,  
80 East Concord Street,  
Boston, Massachusetts 02118

STEVEN ROSE

Department of Neurosciences,  
Albert Einstein College of Medicine,  
1300 Morris Park Avenue,  
Bronx, New York 10461

Received May 27; accepted June 4, 1975.

- <sup>1</sup> del Castillo, J., and Katz, B., *J. Physiol., Lond.*, **128**, 396-411 (1955).
- <sup>2</sup> Katz, B., *Nerve, Muscle and Synapse* (McGraw-Hill, New York, 1966).
- <sup>3</sup> Dunant, Y., Gautron, J., Israel, M., Lesbats, B., and Manaranche, R., *J. Neurochem.*, **19**, 1987-2002 (1972).
- <sup>4</sup> Molenaar, P. C., Polak, R. L., and Nickolson, V. J., *J. Neurochem.*, **21**, 667-678 (1973).
- <sup>5</sup> Richter, J. A., and Marchbanks, R. M., *J. Neurochem.*, **18**, 705-712 (1971).
- <sup>6</sup> Tauc, L., Hoffmann, A., Tsujii, S., Hinzen, D. H., and Faille, L., *Nature*, **250**, 496-498 (1974).
- <sup>7</sup> Heuser, J., and Reese, T., *J. Cell Biol.*, **57**, 315-344 (1973).
- <sup>8</sup> Holtzman, E., and Peterson, E. R., *J. Cell Biol.*, **40**, 863-869 (1969).
- <sup>9</sup> Ceccarelli, B., Hurlbut, W. P., and Mauro, A., *J. Cell Biol.*, **57**, 499-524 (1973).
- <sup>10</sup> Fatt, P., *J. Physiol., Lond.*, **114**, 408-422 (1950).
- <sup>11</sup> Betz, W., and Sakmann, B., *J. Physiol., Lond.*, **230**, 673-688 (1973).
- <sup>12</sup> Hall, Z. W., and Kelly, R. B., *Nature new Biol.*, **232**, 62-63 (1971).
- <sup>13</sup> Kreutzberg, G. W., and Kaiya, H., *Histochemistry*, **42**, 233-237 (1974).
- <sup>14</sup> Crevier, M., and Belanger, L. F., *Science*, **122**, 256-257 (1955).

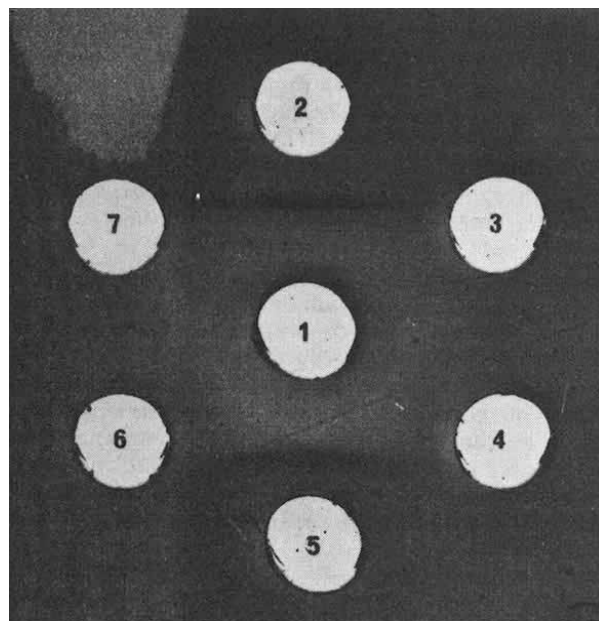
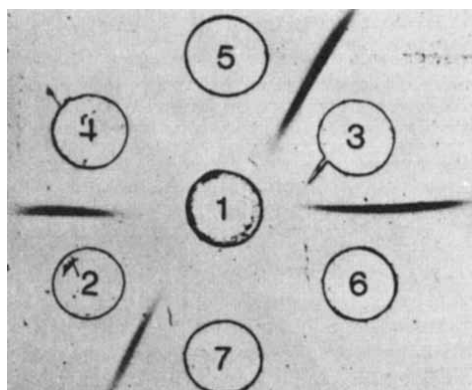
## Differences between detergent-extracted acetylcholine receptor and "cholinergic proteolipid"

SNAKE venom  $\alpha$ -toxins<sup>1</sup> in combination with reversible<sup>2,3</sup> or alkylating<sup>4,5</sup> cholinergic ligands give a selective labelling of the cholinergic (nicotinic) receptor protein (AChR)<sup>6-8</sup>. Since this protein is membrane-bound and therefore expected to possess hydrophobic properties, two different methods have been used for its dissolution and purification: (1) chloroform-methanol extraction<sup>9</sup> and (2) non-denaturing detergent extraction in aqueous media<sup>2,3</sup>. The former solubilises the so-called "cholinergic proteolipid" which, still in organic media, binds charged cholinergic ligands<sup>9</sup>. The latter procedure has made possible the purification of the

AChR by affinity chromatography and the study of the binding of the above-mentioned ligands in physiological media (for reviews see refs 6–8). Furthermore, the serum of rabbits immunised against the purified AChR<sup>10–12</sup> blocks *in vivo* the physiological response to cholinergic agonists<sup>10,11</sup>, thereby confirming that the detergent-extracted AChR is indeed involved in the permeability change caused by acetylcholine. On the other hand, the physiological importance of the "cholinergic proteolipid" remains undetermined, though the challenge to the actual purification<sup>13</sup> has recently been refuted<sup>14</sup>. It is therefore the aim of the present work to compare the "cholinergic proteolipid" and the detergent-extracted AChR from the same sources: the electric organs of *Electrophorus electricus* and *Torpedo marmorata* using the selective labels  $\alpha$ -toxin<sup>15</sup> and the covalent affinity reagent 4-(N-maleimido)-phenyltrimethylammonium iodide<sup>4</sup> in combination with immunodiffusion tests<sup>11</sup>.

Frozen electric tissue from *Electrophorus electricus* was thawed, homogenised in water, lyophilised and extracted with chloroform-methanol (2:1 v/v) as described by La Torre *et al.*<sup>16</sup>. The crude organic extract was partitioned with one fifth its volume of water, and the proteolipids of the organic phase precipitated with four volumes of diethyl ether at  $-20^{\circ}\text{C}$ . After standing overnight, the precipitate was redissolved in chloroform-methanol (2:1 v/v), concentrated *in vacuo*, and chromatographed through the organophilic dextran gel Sephadex LH 20 using chloroform-methanol mixtures<sup>16</sup>. The proteolipid fraction III (the so-called "receptor proteolipid")<sup>9,16</sup> was isolated for further study. In other series of experiments, fresh and lyophilised electric tissue were extracted with chloroform-methanol at various temperatures ( $25^{\circ}\text{C}$ ,  $-50^{\circ}\text{C}$ ,  $-80^{\circ}\text{C}$ ) according to ref. 17. After evaporation of the organic solvent at  $+25^{\circ}\text{C}$  or  $-25^{\circ}\text{C}$ , the samples were re-extracted by adding Triton X-100 in *Electrophorus* Ringer's solution (160 mM NaCl, 5 mM KCl, 2 mM  $\text{MgCl}_2$ , 2 mM  $\text{CaCl}_2$ , 2.5 mM Na phosphate buffer pH 7) to a final 1% detergent concentration and stirred for 1 h at room temperature. The detergent extracted fractions were analysed for their binding activity with  $^3\text{H}$ - $\alpha$ -Naja nigricollis toxin (15 Ci  $\text{mmol}^{-1}$ ) (ref. 15) and for their ability to react with rabbit antibodies raised against purified acetylcholine receptor from *Electrophorus* (anti-AChR), using the Ouchterlony immunodiffusion test<sup>11</sup> (Fig. 1). The Triton X-100 extracted AChR was purified from *Electrophorus* membrane fragments by affinity chromatography<sup>18</sup>.

**Fig. 1** Ouchterlony double diffusion in 1% agarose gel and 0.025 M Veronal buffer pH 8.2. 1, Triton X-100 extract of proteolipid fraction III (ref. 16); 2, 3, AChR purified by affinity chromatography in the presence of Triton X-100 (ref. 18); 4, 5, 6, 7, Anti-AChR sera raised in four different rabbits. The presence of the Triton X-100-extract of proteolipid fraction III in hole No. 1 does not induce any deviation of the precipitation lines between AChR and anti-AChR: no cross reaction can be seen between AChR and the proteolipid fraction III.



**Fig. 2** Ouchterlony double diffusion in 1% agarose gel and 0.023 M Veronal buffer pH 8.2. 1, Anti-proteolipid fraction III sera, raised in two different rabbits; 2, 5, Proteolipid fraction III in 1% Triton X-100; 3, 4, 6, 7, AChR purified by affinity chromatography in 1% Triton X-100. Precipitation lines are seen between hole 1 and holes 2 and 5. The presence of AChR in holes 3, 4, 6 and 7 induces no deviation of the precipitation lines: no cross reaction occurs between AChR and rabbit anti-proteolipid fraction III sera.

Rabbit antisera were also raised against proteolipid peak III from *Electrophorus electricus* electric tissue. Three rabbits each received 500  $\mu\text{g}$  proteolipid peak III in 0.5 ml Freund's adjuvant; the dose was repeated after one week and after a further 2 weeks. The ability of the rabbit antibodies to react with proteolipid peak III and with purified AChR was tested using the Ouchterlony immunodiffusion test<sup>11</sup> (Fig. 2).

Regardless of the temperature of extraction<sup>17</sup> and subsequent concentration, the proteolipid protein transferred to 1% Triton X-100 or emulsified in water, before or after chromatography on Sephadex LH 20, does not show binding of  $^3\text{H}$ - $\alpha$ -toxin nor does it react against anti-AChR antisera. Similarly, no cross reaction between the AChR<sup>18</sup> and the organic extract or the purified "cholinergic proteolipid" of La Torre *et al.*<sup>16</sup> was observed in these immunodiffusion tests.

In another series of experiments 25  $\mu\text{g}$  of the AChR protein purified by affinity chromatography<sup>18</sup> (2,400 nmol  $^3\text{H}$ - $\alpha$ -toxin bound per g of protein) was incubated overnight with  $\text{Cl}_3\text{CH}-\text{CH}_2\text{OH}$  (2:1 v/v) at  $0^{\circ}\text{C}$ ,  $4^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ . After evaporation of the organic solvent with  $\text{N}_2$ , the residual aqueous samples were assayed for  $\alpha$ -toxin binding and immunoprecipitation. In all cases, the ability to bind the cholinergic ligand and to react against the anti-AChR antisera were lost. The immunological and the toxin binding assays on water-solubilised proteolipids might be negative because of a possible partial denaturation of the so-called "receptor proteolipid" during transfer to aqueous solvents, although the detergent-solubilised proteolipid retains its antigenicity to rabbit anti-proteolipid peak III serum as seen in Fig. 2. The AChR was therefore covalently labelled, before its extraction with organic solvents, with 4-(N-maleimido)-phenyltri- $^3\text{H}$ -methylammonium ( $^3\text{H}$ -MPTA), by a procedure similar to that used by Karlin and Cowburn<sup>5</sup> with 4-(N-maleimido)-benzyltri- $^3\text{H}$ -methylammonium ( $^3\text{H}$ -MBTA). This allowed the AChR to be followed



**Table 1**  $^3\text{H}$ -MPTA labelling of AChR-rich membrane fragments from *T. marmorata* (800 nmol  $^3\text{H}$ - $\alpha$ -toxin bound per g of protein)

| $^3\text{H}$ -MPTA                                                              |                                         |
|---------------------------------------------------------------------------------|-----------------------------------------|
| In the absence of $\alpha$ -toxin                                               | 2,400 c.p.m. $\mu\text{g}^{-1}$ protein |
| After preincubation with 30 $\mu\text{g ml}^{-1}$ of unlabelled $\alpha$ -toxin | 8 c.p.m. $\mu\text{g}^{-1}$ protein     |
| CM extraction of labelled membrane fragments                                    |                                         |
| (1) From aqueous phase (15 $\mu\text{g}$ protein)                               |                                         |
| CM extract                                                                      | 50 c.p.m.                               |
| Aqueous phase                                                                   | 34,600 c.p.m.                           |
| (2) From lyophilised membranes (48 $\mu\text{g}$ protein)                       |                                         |
| CM extract                                                                      | 2,500 c.p.m.                            |
| Solid residue                                                                   | 110,000 c.p.m.                          |

Chloroform-methanol (2:1 v/v) (CM) extraction of labelled membrane fragments: the receptor-rich membrane fragments from the electric organ of *Torpedo marmorata* were prepared according to ref. 19. They were then labelled with  $^3\text{H}$ -MPTA following the procedure of Karlin<sup>6</sup>. The receptor-rich membrane fragments (800 nmol  $\alpha$ -toxin sites per g of protein) were incubated for 10 min in the presence of 1 mM dithiothreitol in buffer I (0.1 M NaCl, 1 mM EDTA, 0.01 M Tris-HCl, pH 8). This reduction was stopped by adding 0.5 M Na Phosphate buffer pH 6.7 and cooling to 0 °C. After centrifugation at 100,000g for 90 min, the pellet was resuspended in buffer II (0.15 M NaCl, 1 mM EDTA, 0.01 M Na phosphate buffer pH 7.0). An aliquot was incubated for 90 min at 20 °C with an excess of  $\alpha$ -toxin from *Naja nigricollis*, the rest being incubated without  $\alpha$ -toxin at the same temperature. The two samples were then run in parallel. They were labelled with  $^3\text{H}$ -MPTA (about 2 Ci mmol<sup>-1</sup>) for 2 min at 25 °C. The labelling was stopped with 2-mercaptoethanol. The membrane fragments were then washed by threefold centrifugation followed by resuspension of the pellet in buffer III (0.15 M NaCl, 1 mM EDTA, 0.01 M sodium phosphate buffer, pH 7.0). (1) The labelled membrane fragments were extracted with CM. The extract was partitioned with  $\text{H}_2\text{O}$  and the "theoretical upper phase"<sup>16</sup> was prepared with  $\text{Cl}_3\text{CH} : \text{CH}_3\text{OH} : \text{H}_2\text{O}$  (3:48:47 by volume) and separated by centrifugation (3,000g, 10 min). This procedure was done three times and the radioactivity was measured in the combined upper phases and in the washed CM extract. (2) The membrane fragments were first lyophilised and then extracted with CM, and filtered. The radioactivity was measured in the residue and the CM extract.

even in a denatured state. Table 1 shows the results of the affinity labelling with  $^3\text{H}$ -MPTA of the AChR-rich membrane fragments from *Torpedo marmorata* prepared according to ref. 19.

The native membrane fragments bound 800 nmol of  $^3\text{H}$ - $\alpha$ -toxin per g of protein; after reduction by dithiothreitol, 2,400 c.p.m. of  $^3\text{H}$ -MPTA (about 2 Ci mmol<sup>-1</sup>) were covalently attached per  $\mu\text{g}$  of protein. Preincubation of the membrane fragments with 30  $\mu\text{g ml}^{-1}$   $\alpha$ -toxin before affinity alkylation reduces by 99.7% the amount of  $^3\text{H}$ -MPTA retained by the membrane fragments confirming that, under these conditions, the labelling of  $^3\text{H}$ -MPTA is specific for the AChR site.

Samples of the  $^3\text{H}$ -MPTA labelled membrane fragments were extracted with chloroform-methanol (2:1 v/v) and the crude extract extensively washed with  $\text{H}_2\text{O}$  (Table 1). No radioactivity was recovered in the organic phase. All the radioactivity was recovered in the combined aqueous phases and in a precipitate which formed at the water-chloroform-methanol interface. When the chloroform-methanol (2:1 v/v) extraction was performed on lyophilised tissue, as described by La Torre *et al.*<sup>16</sup>, only 1.7% of the radioactivity was found in the organic extract, the rest being recovered in the residue.

The experiments using affinity alkylation of the membrane-bound AChR from *Torpedo marmorata*, in coincidence with those carried out on the material extracted from *Electrophorus electricus*, indicate that in agreement with Karlin<sup>7</sup> the AChR subunit(s) carrying the site(s) for binding  $\alpha$ -toxin or MPTA and recognised by the anti-AChR antiserum<sup>10-12</sup> are not extracted with chloroform-methanol (2:1 v/v). The organic-extracted "cholinergic" proteolipids<sup>9,16,17</sup> from the same sources are therefore different

from the detergent-extracted AChR in all these respects.

FJB and GGL were recipients of short-term EMBO Fellowships during their stay in Paris. Research supported by The Collège de France, CNRS, DGRST CEA and NIH.

F. J. BARRANTES

Max-Planck Institut für biophysikalische Chemie,  
3400 Göttingen-Nikolausberg, West Germany

J. P. CHANGEUX

Neurobiologie Moléculaire,  
Institut Pasteur,  
Paris, France

G. G. LUNT

Biochemistry Department,  
University of Bath, UK

A. SOBEL

Neurobiologie Moléculaire,  
Institut Pasteur,  
Paris, France

Received March 25; accepted June 6, 1975.

- Lee, C. Y., *Clinical Toxicology*, **3**, 457-472 (1970).
- Changeux, J. P., Kasai, M., and Lee, C. Y., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1241-1247 (1970).
- Miledi, R., Molinoff, P., and Potter, L. T., *Nature*, **229**, 554-557 (1971).
- Karlin, A., and Winnik, M., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 668-674 (1968).
- Karlin, A., and Cowburn, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3636-3640 (1973).
- Cohen, J. B., and Changeux, J. P., *A. Rev. Pharmac.*, **15**, 83-103 (1975).
- Karlin, A., *Life Sci.*, **14**, 1385-1415 (1974).
- Rang, H. P., *Q. Rev. Biophys.*, **7**, 283-399 (1975).
- De Robertis, E., *Science*, **171**, 963-971 (1971).
- Patrick, J., and Lindstrom, J., *Science*, **180**, 871-872 (1973).
- Sugiyama, H., Benda, P., Meunier, J. C., and Changeux, J. P., *FEBS Lett.*, **35**, 124-128 (1973).
- Heilbronn, E., and Mattson, C., *J. Neurochem.*, **22**, 315-317 (1974).
- Levinson, S. R., and Keynes, R. D., *Biochim. biophys. Acta*, **288**, 241-247 (1972).
- Donellan, J. F., and Cattell, K. J., *Biochem. Soc. Trans.*, **3**, 106-109 (1975).
- Menez, A., Morgat, J. L., Fromageot, P., Ronseray, A. M., Boquet, P., and Changeux, J. P., *FEBS Lett.*, **17**, 333-335 (1971).
- La Torre, J. L., Lunt, G. S., and de Robertis, E., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 716-720 (1970).
- Izumi, F., and Freed, S., *FEBS Lett.*, **41**, 151-155 (1974).
- Meunier, J. C., Sealock, R., Olsen, R., and Changeux, J. P., *Eur. J. Biochem.*, **45**, 371-394 (1974).
- Cohen, J. B., Weber, M., Huchet, M., and Changeux, J. P., *FEBS Lett.*, **26**, 43-47 (1972).

## Possible mechanism for action of prolactin on mammary cell sodium transport

PROLACTIN, like other polypeptide and glycoprotein hormones from the pituitary gland, exerts its effects on target cells through interaction with receptors on the plasma membrane<sup>1-4</sup>. Unlike several other pituitary hormones, this does not occur through activation of adenylyl cyclase<sup>5</sup>, and the mechanism of action of prolactin must therefore lie elsewhere.

In this study we have investigated a possible mechanism which is derived from the earlier (in evolution), osmoregulatory function of prolactin. In estuarine fishes, prolactin maintains internal osmolarity by regulating  $\text{Na}^+$  diffusion across the gills, and by controlling the activity of  $\text{Na}^+/\text{K}^+$ -activated ATPase, the active monovalent ion pump, in gills and kidney<sup>6</sup>. Prolactin may therefore act similarly on mammary alveolar cells, which are of epithelial origin, through the regulation of monovalent cation transport.

Measurement of sodium<sup>22</sup> transport in response to prolactin, growth hormone and albumin was carried out using slices of lactating mammary tissue incubated with  $^{22}\text{Na}^+$  as described in Table 1. The results of these experiments show a highly significant decrease in tissue  $^{22}\text{Na}^+$  after addition of prolactin, and a less significant decrease after growth hormone. The growth hormone preparation had less than 0.5 IU per mg prolactin potency, but it is possible that this was sufficient to cause the decrease.

In further experiments, incubation of mammary tissue slices in the presence of  $5 \times 10^{-5}$  M ouabain resulted in a 10% in-

crease in tissue  $^{22}\text{Na}^+$  content. Incubation with ouabain plus prolactin abolished the decrease in  $^{22}\text{Na}^+$  observed after prolactin alone, and resulted in tissue  $^{22}\text{Na}^+$  content similar to incubations with ouabain alone.

In these experiments, tissue  $^{22}\text{Na}^+$  content after incubation would be caused by  $^{22}\text{Na}^+$  entry by diffusion, opposed by the activity of the  $\text{Na}^+$  pump ( $\text{Na}^+/\text{K}^+$ -activated ATPase) in extruding intracellular  $\text{Na}^+$ . Ouabain selectively inhibits this  $\text{Na}^+$  pump<sup>8</sup>, and was used in these experiments to demonstrate whether the reduced  $^{22}\text{Na}^+$  content of the tissue caused by prolactin occurred as a result of lowered  $\text{Na}^+$  entry or accelerated  $\text{Na}^+$  extrusion. As ouabain abolished the prolactin effect, the data suggest that the action of prolactin is on the  $\text{Na}^+$  pump to increase  $\text{Na}^+$  transport out of the cell.

Monovalent ion concentrations in lactating mammary tissue were measured after incubation in the presence or absence of prolactin. With rabbit tissue (Table 2), and with guinea pig tissue (I.R.F. and J.M.R., unpublished), significantly lower tissue  $\text{Na}^+$  concentrations resulted from incubation with prolactin. Tissue  $\text{K}^+$  concentrations did not change significantly, but tissue  $\text{Cl}^-$  was significantly decreased after incubation with prolactin. Because of the permeability of the apical membranes of the cells to  $\text{K}^+$  and the high concentration gradient to be expected between intra- and extracellular  $\text{K}^+$  in this system, any changes in cell  $\text{K}^+$  transport may well be masked.

The observed decrease in tissue  $\text{Na}^+$  when prolactin is present in the incubation indicates that prolactin directly stimulates the transport of  $\text{Na}^+$  out of the cell, presumably by increasing the activity of  $\text{Na}^+/\text{K}^+$ -activated ATPase. To confirm this, we are at present investigating the influence of prolactin on isolated mammary membrane fragments having  $\text{Na}^+/\text{K}^+$ -activated ATPase activity. Note that cytological studies have located both the  $\text{Na}^+/\text{K}^+$ -activated ATPase and the prolactin receptors on the same restricted region of the alveolar cell membrane<sup>2,10</sup>. The studies of Cowie<sup>11</sup> on milk composition in the rabbit provide supporting evidence for the effect of prolactin on monovalent ion transport, as he showed a rise in milk  $\text{Na}^+$  and  $\text{Cl}^-$  in late lactation, which could be reversed by prolactin. As the ionic composition of milk reflects alveolar intracellular ionic composition<sup>12</sup>, it is likely that monovalent cation concentration in the milk is regulated by ion transport at the basal membrane of the cell, and thus would be responsive to prolactin.

It seems likely therefore that an early response of mammary alveolar cells to prolactin is the activation of the  $\text{Na}^+$  pump leading to a decreased intracellular  $\text{Na}^+$  concentration, and a

**Table 2** Monovalent ion concentrations in slices of lactating mammary alveolar tissue from rabbits after incubation with increasing concentrations of prolactin

| Prolactin concentration<br>( $\text{M} \times 10^{-8}$ ) | Monovalent ion concentration<br>( $\text{meq kg}^{-1}$ tissue; mean $\pm$ s.e.m.) |                |                          |
|----------------------------------------------------------|-----------------------------------------------------------------------------------|----------------|--------------------------|
|                                                          | $\text{Na}^+$                                                                     | $\text{K}^+$   | $\text{Cl}^-$            |
| 0.00                                                     | $114.1 \pm 2.1$                                                                   | $23.7 \pm 0.9$ | $90.2 \pm 2.7$           |
| 4.35                                                     | $107.9 \pm 2.2^*$                                                                 | $25.3 \pm 1.2$ | $78.3 \pm 4.2^{\dagger}$ |
| 8.70                                                     | $104.8 \pm 3.4^{\dagger}$                                                         | $22.9 \pm 1.0$ | $82.5 \pm 2.7^*$         |
| 17.40                                                    | $105.0 \pm 2.2^{\dagger}$                                                         | $25.1 \pm 1.1$ | $84.0 \pm 3.0$           |

\* $P < 0.05$ ;  $^{\dagger}P < 0.01$ .

Lactating rabbits were used on three separate occasions for these experiments and the data combined. Each rabbit was treated with bromocriptin as described in Table 1. Each experiment comprised 20 samples of 500 mg 0.7 mm slices of mammary tissue, incubated in Krebs bicarbonate buffer, pH 7.4, for 1 h with continuous bubbling of 95%  $\text{O}_2$ -5%  $\text{CO}_2$ . Prolactin was added to the incubation medium in three sets of five samples in each experiment at the concentrations shown, which correspond to 0.05, 0.1 and 0.2  $\mu\text{g}$  per mg tissue. After incubation, the tissue was homogenised with an overhead homogeniser (Ultra-Turrax, Type TP1810 Janke and Kunkel K.G., Staufen i. Breisgau) in 4 ml deionised water. Monovalent ion content was measured using an Atomic Absorption Spectrophotometer (AA100, Techtron, Melbourne, Australia) and the chloride content determined by titration<sup>9</sup>.

marked change in the intracellular  $\text{Na}^+/\text{K}^+$  ratio. These changes may act as an intracellular messenger in the biosynthetic events resulting from prolactin stimulation, as alterations in monovalent cation concentrations have been shown to stimulate nuclear activity in insect salivary glands in a hormone-like response<sup>13</sup>. Investigations of mammary alveolar cell responses to changes in monovalent cation concentrations may thus shed light on the mechanism of action of prolactin.

We thank the Nuffield Foundation for support, and Mr T. Buckley and Mrs K. Tadros for assistance.

IAN R. FALCONER  
J. M. ROWE

Department of Biochemistry and Nutrition,  
University of New England, Armidale,  
New South Wales, Australia 2351

Received April 21; accepted June 11, 1975.

- Turkington, R. W., *Biochem. biophys. Res. Commun.*, **41**, 1362-1367 (1970).
- Birkinshaw, M., and Falconer, I. R., *J. Endocr.*, **55**, 323-334 (1972).
- Shiu, R. P. C., and Friesen, H. G., *J. biol. Chem.*, **249**, 7902-7911 (1974).
- Shiu, R. P. C., and Friesen, H. G., *Biochem. J.*, **140**, 301-311 (1974).
- Turkington, R. W., in *Lactogenic Hormones* (edit. by Wolstenholme, G. E. W., and Knight, J.), 111-127 (Churchill-Livingstone, Edinburgh and London, 1972).
- Ensor, D. M., and Ball, J. N., *Fedn Proc.*, **31**, 1615-1623 (1972).
- Hart, I. C., *J. Endocr.*, **57**, 179 (1973).
- Skou, J. C., *Physiol. Rev.*, **45**, 596-617 (1965).
- Cotlove, E., Trantham, H. V., and Bowman, R. L., *J. Lab. clin. Med.*, **31**, 461-468 (1958).
- Kinura, T., *Japan Obstet. Gynec. Soc.*, **21**, 301-308 (1969).
- Cowie, A. T., *J. Endocr.*, **44**, 437-450 (1969).
- Linzell, J. M., and Peaker, J. L., *Physiol. Rev.*, **51**, 564-597 (1971).
- Kroeger, H., in *Endocrine Genetics* (edit. by Spickett, S. G.), *Endocr. Soc. Symp.*, **15**, 55-66 (Cambridge University Press, Cambridge, 1967).

**Table 1** Effect of prolactin, growth hormone or albumin on  $^{22}\text{Na}^+$  content of mammary gland slices

| Treatment      | Concentration<br>( $\mu\text{g}$ protein<br>per mg tissue) | $^{22}\text{Na}^+$ content of 0.1 g slices<br>(c.p.m. $\pm$ s.e.m.) (3 experiments,<br>6 replicates in each) |
|----------------|------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| Control        | —                                                          | $376.2 \pm 16.5$                                                                                             |
| Prolactin      | 0.5                                                        | $326.8 \pm 13.3^{\dagger}$                                                                                   |
| Growth hormone | 0.5                                                        | $342.6 \pm 13.8^*$                                                                                           |
| Albumin        | 0.5                                                        | $381.1 \pm 18.5$                                                                                             |

\* $P \approx 0.05$ ;  $^{\dagger}P < 0.01$ .

Lactating mammary alveolar tissue was obtained from rabbits 10-20 d post partum, killed by cervical dislocation with minimum disturbance. For 3 d before killing, bromocriptin (CB154, gift from Professor E. Flukiger and Dr H. Friedle, Sandoz, Basle) was administered daily at 1 mg  $\text{kg}^{-1}$  subcutaneously, to inhibit prolactin secretion<sup>7</sup>. The mammary alveolar tissue was cooled, chopped into 0.7 mm slices (McIwain chopper), and washed in Tyrode's solution (pH 7.4) at 0 °C before use. Prolactin (NIH P/S9), growth hormone (NIH B 17GH gifts from the National Institutes of Health) and bovine serum albumin (Commonwealth Serum Laboratories, Melbourne) dissolved in Tyrode's solution were used as described. To measure  $^{22}\text{Na}^+$  transport, 0.1 g tissue slices were incubated in 2 ml Tyrode's solution containing 1% rabbit serum at 37 °C for 2 h in an oscillating (200 cycles  $\text{min}^{-1}$ ) water bath, in the presence of 0.1  $\mu\text{Ci}$   $^{22}\text{Na}^+$ . To this incubation medium were added 20  $\mu\text{g}$  prolactin, growth hormone or albumin. After incubation the supernatant was decanted, the slices washed at 0 °C for 15 min in Tyrode's solution, blotted and  $^{22}\text{Na}^+$  content determined in a well-type scintillation counter.

## Internal regulation of iron absorption

MCCANCE and Widdowson<sup>1</sup> concluded that body iron content is regulated by variation in the amount absorbed and not by variation in excretion, and many workers have since attempted to define the factors which relate iron absorption to the needs of the body. Although there is a large body of data on the intracellular mechanisms of absorption<sup>2</sup> this has not advanced our understanding of the physiology of regulation.

There are three main theories describing the regulation of iron absorption. The first proposes that this control is exercised mainly by the gut mucosa. The epithelial cells of the small intestine incorporate iron from the plasma during their development, and it has been suggested that the amount taken up by these cells reflects the iron status of the body and that this in turn conditions them to allow the passage of more or less iron<sup>3</sup>. Within the cell this



control may be related to the cell's ability to synthesise ferritin<sup>3</sup> (which may retain iron and so prevent the transfer of unwanted iron to the plasma), to the degree of saturation of a hypothetical carrier protein<sup>3,4</sup> or to the iron requirements of the cell for haem synthesis<sup>5</sup>. Changes in the rate of plasma iron turnover have, however, been shown to affect iron absorption and it has been thought that this, rather than changes in plasma iron concentration, may be the main determinant of the iron status of the developing epithelial cell<sup>6</sup>.

The second, related, theory proposes that control is a function of the different characteristics of the two iron-binding sites of the transferrin molecule in the plasma<sup>7</sup>. The number of molecules with two iron atoms will, it is assumed, reflect the level of body iron stores and determine the concentration of iron in the mucosal cells. This may then regulate the transfer from the mucosa to the plasma as in the first theory. It is suggested, however, that one transferrin iron-binding site is preferentially relieved of iron by the red cell precursors of the marrow and is also particularly avid for intestinal iron. This superimposes a second controlling factor on iron absorption whereby it may be related to the demands of erythropoiesis as well as to the level of the iron stores<sup>7</sup>.

The third theory involves a humoral factor which is secreted either in direct or inverse proportion to body iron requirements and which signals the gut to increase or decrease iron absorption. Conrad<sup>8</sup> has commented on a number of experiments exploring this hypothesis.

There is no direct experimental evidence to support either the transferrin or the humoral control theories. The mucosal mechanisms which have been proposed do not explain all the observed phenomena and indeed recent observations have cast doubt on some of the earlier conclusions. These were based on measurements of the iron content of the whole gut wall and seemed to show an inverse relationship between mucosal iron and iron absorption<sup>6,9</sup>. Direct measurement of the iron content of epithelial cell homogenates has now shown that there is no direct relationship between epithelial cell iron and absorption<sup>10-12</sup>. Moreover, iron-deficient epithelial cells have a capacity for ferritin synthesis equal to that of normal cells<sup>13</sup>. The iron or ferritin content of the mucosal cell does not seem to be the controlling factor in iron absorption. The basic question remains — how is iron absorption induced to respond to the rate of erythropoiesis and the level of iron stores? We suggest that this regulation may best be explained by a consideration of internal iron exchange.

Iron circulating in the plasma bound to transferrin exchanges with iron in all cells of the body. An exchangeable iron pool may be defined as iron available for binding by circulating transferrin irrespective of its site or chemical form. Its size will depend not only on cellular iron concentration but also on the transferrin-binding sites in different tissues. The probability of an iron atom from a given tissue being picked up by a free receptor site on a transferrin molecule is proportional to the ratio of exchangeable iron in that tissue to the exchangeable iron in the whole body. The number of iron atoms picked up in this way per unit time is equal to the number cleared from the plasma. This is reflected in the experimentally measured plasma iron turnover. Iron transfer from the intestinal epithelium to the plasma is therefore proportional to

$$\frac{\text{Intestinal exchangeable iron}}{\text{Total exchangeable iron}} \times \text{Plasma iron turnover}$$

In general the total exchangeable iron in the body is directly related to the level of storage iron and our hypothesis explains why low iron stores are associated with increased iron absorption and high stores with reduced

absorption. It predicts that this relationship will hold not only in patients with iron deficiency or iron overload but also in normal subjects; this has been observed<sup>14,15</sup>. This hypothesis also shows how an increase in iron absorption may follow an increase in plasma iron turnover as a result of erythroid overactivity. It also explains why this is not always found. When increased erythropoiesis is associated with haemolysis, increased haemoglobin catabolism results in an increased amount of iron in the reticuloendothelial system. This increases the total exchangeable iron and will counterbalance the effect of plasma iron turnover on iron absorption, and thus a stable haemolytic anaemia may be associated with normal iron absorption<sup>16</sup>.

In conclusion, the present hypothesis offers a simple rational explanation of the relationship between several endogenous factors which can influence iron absorption, and provides a framework for further investigation.

I. CAVILL  
M. WORWOOD  
A. JACOBS

Department of Haematology,  
Welsh National School of Medicine,  
Cardiff, UK

Received April 1; accepted June 11, 1975.

- <sup>1</sup> McCance, R. A., and Widdowson, E. M., *Lancet*, ii, 680-684 (1937).
- <sup>2</sup> Turnbull, A., in *Iron in Biochemistry and Medicine* (edit. by Jacobs, A., and Worwood, M.), 369-403 (Academic, London and New York, 1974).
- <sup>3</sup> Crosby, W. H., *Blood*, 22, 441-449 (1963).
- <sup>4</sup> Pinkerton, P. H., *Ann. Int. Med.*, 70, 401-408 (1969).
- <sup>5</sup> Jacobs, A., in *Clinics in Haematology*, 2, no. 2, (edit. by Callender, S. T.), 323-337 (Saunders, London 1973).
- <sup>6</sup> Weintraub, L. R., Conrad, M. E., and Crosby, W. H., *Blood*, 24, 19-24 (1964).
- <sup>7</sup> Fletcher, J., and Huehns, E. R., *Nature*, 218, 1211-1214 (1968).
- <sup>8</sup> Conrad, M. E., *Gastroenterology*, 57, 225-228 (1969).
- <sup>9</sup> Conrad, M. E., Weintraub, L. R., and Crosby, W. H., *J. clin. Invest.*, 43, 963-974 (1964).
- <sup>10</sup> Balcerzak, S. P., and Greenberger, N. J., *Nature*, 220, 270-271 (1968).
- <sup>11</sup> Richmond, V. S., Worwood, M., and Jacobs, A., *Br. J. Haemat.*, 14, 605-614 (1972).
- <sup>12</sup> Mattii, R., Mielke, C. H., Levine, P. H., and Crosby, W. H., *Blood*, 42, 959-966 (1973).
- <sup>13</sup> Brittin, G. M., and Raval, D., *J. Lab. clin. Med.*, 75, 811-817 (1970).
- <sup>14</sup> Cook, J. D., Lipschitz, D. A., Miles, L. E. M., and Finch, C. A., *Am. J. clin. Nutr.*, 27, 681-687 (1974).
- <sup>15</sup> Walters, G. O., Jacobs, A., Worwood, M., Trevett, D., and Thomson, W., *Gut*, 16, 188-192 (1975).
- <sup>16</sup> Crosby, W. H., in *Regulation of Hematopoiesis*, 1 (edit. by Gordon, A. S.), 519-537 (Appleton-Century-Crofts, New York, 1970).

## Appearance of hypoxanthine guanine phosphoribosyltransferase activity as a consequence of mycoplasma contamination

MYCOPLASMAS are common contaminants of cells in culture and are often responsible for profound alterations in the metabolism of infected cells<sup>1,2</sup>. Such alterations include chromosomal aberrations<sup>3,4</sup> and changes in host cell enzyme activities<sup>5</sup>. We have reported that amniotic fluid cell cultures contaminated with mycoplasmas revealed a significant increase in chromosomal aberrations over those seen in uninfected cultures<sup>6</sup>. This finding demonstrated that the presence of these organisms in amniotic fluid cell lines poses a potential hazard to prenatal chromosome diagnosis.

In addition to chromosomal disorders many inherited metabolic disorders can now be diagnosed prenatally, including biochemical defects involving diminished or complete absence of enzyme activities<sup>7</sup>. The studies reported here were designed to determine whether cells lacking a specific enzyme activity may acquire significant levels of activity as a consequence of mycoplasma infection. The cell line chosen for these studies was D98/AH-2, a human cell line which lacks measurable hypoxanthine guanine phosphoribosyltransferase (HGPRT) activity<sup>8</sup>. HeLa cells were used as a positive control for human HGPRT activity.

D98/AH-2 and HeLa cells were grown in Eagle's basal medium supplemented with 10% calf serum, 28 mM HEPES buffer and 100 U ml<sup>-1</sup> penicillin and subcultured

twice weekly. Actively dividing D98/AH-2 cells were infected with  $1 \times 10^6$  colony forming units (CFU) of *Acholeplasma laidlawii* or *Mycoplasma hyorhinis*, two common contaminants of cell cultures<sup>1,2</sup>. The infected cells were assayed for HGPRT activity 7 d and 28 d after infection. The HGPRT activities of the two mycoplasma strains were assayed separately.

Representative HGPRT activities are shown in Table 1. Pellets of both *A. laidlawii* and *M. hyorhinis* contained high levels of HGPRT activity. D98/AH-2 cells, as expected, had no measurable activity. Significant levels of HGPRT activity were however found in the infected cultures. In particular, cells infected with *M. hyorhinis* had levels of activity similar to that of the HeLa control. The levels of HGPRT activity probably represent a conservative estimate of the actual activity since 5–40% of inosinic acid was apparently converted to inosine and was not included in our calculated activities. This was probably due primarily to mycoplasmal 5'-nucleotidase activity<sup>10</sup>, although D98/AH-2 5'-nucleotidase activity may have been a contributory factor. It is of interest to note that cells infected with *M. hyorhinis* had much higher levels of enzyme activity than did cells infected with *A. laidlawii*, whereas both mycoplasma strains alone have comparable activity.

An attempt was made to correlate activity with a degree of contamination by measuring the CFUs of mycoplasmas in the cell pellets. This provides a relatively crude estimate of the number of viable mycoplasmas<sup>11</sup>, but it permits the inference that the low HGPRT activities in cells infected with *A. laidlawii* were due to lower mycoplasma: cell ratios than those cultures infected with *M. hyorhinis*. This is further supported by the fact that no inhibition of mycoplasmal HGPRT activity was seen when D98/AH-2 cells were mixed with high numbers of *A. laidlawii* just before the HGPRT assay (Fig. 1), indicating that D98/AH-2 cell extract was not inhibitory to the *Acholeplasma* HGPRT. In a separate series of experiments hypoxanthine was added to cell cultures at the time of infection in an attempt to induce higher levels of mycoplasmal HGPRT activity. As shown in Table 1 no significant increase was noted in D98/AH-2 culture infected with *M. hyorhinis*. The apparent increase in HGPRT activity in cells infected with

*A. laidlawii* in the presence of hypoxanthine may merely represent the inaccuracy of the quantitative assay of viable mycoplasmas associated with the cells.

Electrophoretic analysis of mycoplasmal and cell extracts was done to compare the mobilities of the mycoplasmal and human HGPRT enzymes. Slab gel electrophoresis of infected cells showed bands of activity only for those infected with *M. hyorhinis* (Fig. 1). Distinct bands due to *A. laidlawii* activity were seen only when these mycoplasmas and uninfected cells were mixed together just before extraction. Both mycoplasmal HGPRT activities migrate to a position distinct from that of human HGPRT.

We wish to draw notice to the extra band arrowed in the HeLa cell profile in Fig. 1. These cells were obtained from a laboratory subsequently shown to possess mycoplasma contaminated cell lines (E.J.S. and J.T., unpublished observations). Unfortunately this particular cell line was lost before routine mycoplasma testing was performed. All subsequent electrophoretic analyses of HeLa HGPRT activity using cells known to be free of mycoplasma did not, however, reveal this band.

Our results clearly indicate that the presence of mycoplasmas as contaminants of cell cultures can lead to altered enzyme patterns. This finding is of considerable importance since it suggests that errors in prenatal diagnosis may occur if amniotic fluid cell cultures are contaminated with mycoplasmas. Of particular relevance is the prenatal diagnosis of the Lesch-Nyhan syndrome in which HGPRT activity is diminished<sup>12</sup>. We have also demonstrated significant levels of hexosaminidase A activity in mycoplasmas (E.L.S., J. Tallman, and E.J.S., unpublished observations); the enzyme which is used for pre- and postnatal diagnosis of Tay-Sachs disease<sup>13</sup>. Mycoplasmas also express adenine phosphoribosyltransferase activity at levels higher than those observed in cultured human cells when calculated on a total protein basis (J.A.T. and E.J.S., unpublished observations).

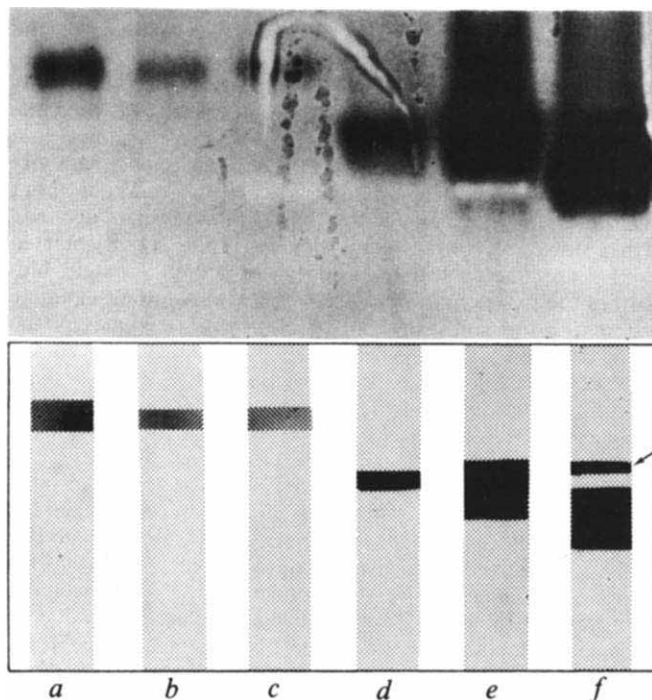
The degree of mycoplasma contamination reached in our deliberately infected cultures is comparable with levels encountered in accidentally contaminated cell lines. Because of the high frequency of mycoplasma contamination found in tissue culture facilities we believe it is

Table 1 HGPRT activities of *A. laidlawii* and *M. hyorhinis* in cell cultures

| Sample                                                                                                | HGPRT activity<br>(c.p.m. in inosinic acid/ $\mu$ g protein/min of assay) | Approximate no. of<br>viable mycoplasmas/0.1 ml<br>packed cell volume ( $\pm 0.5 \log_{10}$ ) |
|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| <i>A. laidlawii</i>                                                                                   | 27.46                                                                     | $3.0 \times 10^9$                                                                             |
| <i>M. hyorhinis</i>                                                                                   | 17.45                                                                     | $1.1 \times 10^9$                                                                             |
| D98/AH-2 cells                                                                                        | No activity                                                               | Negative                                                                                      |
| D98/AH-2 cells infected<br>with <i>M. hyorhinis</i>                                                   | 18.27                                                                     | $1.9 \times 10^8$                                                                             |
| D98/AH-2 cells infected<br>with <i>M. hyorhinis</i> in the<br>presence of $10^{-4}$ M<br>hypoxanthine | 5.58                                                                      | $8.0 \times 10^7$                                                                             |
| D98/AH-2 cells infected<br>with <i>A. laidlawii</i>                                                   | No activity                                                               | $2.0 \times 10^7$                                                                             |
| D98/AH-2 cells infected<br>with <i>A. laidlawii</i> in the<br>presence of $10^{-4}$ M<br>hypoxanthine | 2.87                                                                      | $2.0 \times 10^7$                                                                             |
| HeLa                                                                                                  | 17.75                                                                     | Negative                                                                                      |

Mycoplasmas were grown in broth for 2–4 d at 37 °C, then collected by centrifugation at 10,000 r.p.m. for 20 min, washed twice with phosphate buffered saline (PBS) and the pellets stored at –37 °C until the time of assay. Cell cultures were collected by trypsinisation, washed twice with PBS, pelleted and stored at –70 °C. In one series of experiments  $10^{-4}$  M hypoxanthine was added to the growth medium at the time of infection in an attempt to induce higher levels of mycoplasmal HGPRT activity. Extracts of mycoplasma and cell pellets were prepared by sonication. The assay was started by the addition of the sample extract to 100  $\mu$ l of reaction mixture containing 0.25  $\mu$ Ci <sup>14</sup>C-hypoxanthine (specific activity 5.37 Ci mM<sup>-1</sup>); magnesium chloride 5 mM; 5-phosphoribosyl-1-pyrophosphate 1 mM; Tris buffer (pH 7.4) 55 mM; and bovine serum albumin 150  $\mu$ g. The reaction was carried out at 37 °C and samples removed at 0, 10 and 30 min. The reaction in each case was stopped by the addition of formic acid (to 0.66 M) and immersion in a dry ice-methanol bath. Hypoxanthine, inosine and inosine monophosphate were separated by spotting 5- $\mu$ l samples on Eastman Chromogram Cellulose TLC sheets using non-radioactive markers for identification and 5% Na<sub>2</sub>HPO<sub>4</sub> as a solvent. Spots were visualised with ultraviolet light, cut out and counted by scintillation spectrometry.





**Fig. 1** Separation of mycoplasmal and human HGPRT activities. Electrophoretic analysis was done according to the method of Tischfield *et al.*<sup>9</sup>. The technique involves slab gel electrophoresis followed by lanthanum precipitation of the charged, labelled product and autoradiography of the dried gel. *a*, *M. hyorhina*; *b* and *c*, D98/AH-2 infected with *M. hyorhina*; *d*, *A. laidlawii*; *e*, D98/AH-2 and *A. laidlawii* mixed just before electrophoresis; *f*, HeLa control for human HGPRT. Note the extra band (arrowed) which may be due to accidental mycoplasma contamination (see text).

imperative that prenatal diagnostic centres should institute routine screening of their amniotic fluid cultures for mycoplasmas. Since cultured skin fibroblasts are also used for the postnatal biochemical diagnosis of many inherited metabolic disorders, mycoplasma contamination could interfere with these determinations as well. If further studies show that mycoplasma contaminants of cell cultures have enzyme activities which migrate distinctly from human enzymes by gel electrophoresis, then this procedure could be used to distinguish between their respective activities.

These results should also serve as a warning to investigators involved in somatic cell genetics, where contaminating mycoplasma enzyme activities may be confused with isoenzyme expression<sup>14</sup> and 'reactivation' of enzyme activities after somatic cell hybridisation<sup>15</sup>. A further potential hazard to this area of cell biology is the known capacity of mycoplasmas to induce chromosomal aberrations, including translocations<sup>3,4,6</sup>, which could lead to problems of assigning gene activities to specific chromosomes<sup>16</sup>.

These studies were supported, in part, by grants from the National Institutes of Health and the American Cancer Society. We thank John Trill for technical assistance.

ERIC J. STANBRIDGE

Department of Medical Microbiology,  
Stanford University School of Medicine,  
Stanford, California 94305

JAY A. TISCHFIELD

Department of Biology,  
Case Western Reserve University,  
Cleveland, Ohio 44106

EDWARD L. SCHNEIDER

Laboratory of Cellular and Comparative Physiology,  
NIA, NIH, DHEW and Baltimore City Hospitals,  
Baltimore, Maryland 21224

Received April 21; accepted June 2, 1975.

<sup>1</sup> Stanbridge, E., *Bact. Rev.*, **35**, 206-221 (1971).

<sup>2</sup> Fogh, J., Holmgren, N. B., and Ludovici, P. P., *In Vitro*, **7**, 26-41 (1971).

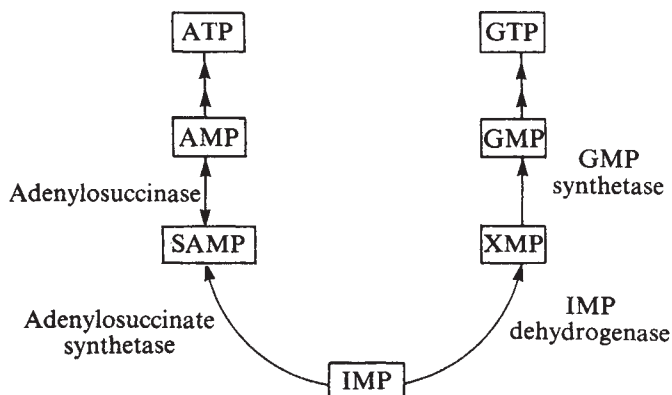
- <sup>3</sup> Paton, G. R., Jacobs, J. P., and Perkins, F. T., *Nature*, **207**, 43-45 (1965).
- <sup>4</sup> Stanbridge, E., Onen, M., Perkins, F. T., and Hayflick, L., *Expl. Cell Res.*, **57**, 397-410 (1969).
- <sup>5</sup> Rose, N. R., Milisauskas, V., and Kite, J. H., Jr, *Proc. Soc. exp. Biol. Med.*, **140**, 390-394 (1972).
- <sup>6</sup> Schneider, E. L., *et al.*, *Science*, **184**, 477-480 (1974).
- <sup>7</sup> Nadler, H. L., *Adv. Human Genet.*, **3**, 1-38 (1972).
- <sup>8</sup> Szybalski, W., Szybalska, E. H., and Ragni, G., *Natn. Cancer Inst. Monogr.*, **7**, 75-89 (1962).
- <sup>9</sup> Tischfield, J. A., Bernhard, H. P., and Ruddie, F. H., *Analyt. Biochem.*, **53**, 545-554 (1973).
- <sup>10</sup> Razin, S., *J. Microbiol.*, **28**, 243-250 (1962).
- <sup>11</sup> Brunner, H., James, W. D., Horswood, R. L., and Chanock, R. M., *J. Immunol.*, **108**, 1491-1498 (1972).
- <sup>12</sup> DeMars, R., Santo, G., Felix, J. S., and Benke, P., *Science*, **164**, 1303-1305 (1969).
- <sup>13</sup> Okada, S., and O'Brien, J. S., *Science*, **165**, 698-700 (1969).
- <sup>14</sup> Harris, H., *Birth Defects*, **9**, 132-137 (1973).
- <sup>15</sup> Bakay, B., Crece, C. M., Koprowski, H., and Nyhan, W. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1998-2002 (1973).
- <sup>16</sup> Tischfield, J. A., and Ruddie, F. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 45-49 (1974).

## IMP dehydrogenase, an enzyme linked with proliferation and malignancy

PREVIOUS studies conducted in this laboratory demonstrated that in a spectrum of liver tumours with different growth rates there is an imbalance in the activities of key enzymes and competing pathways of carbohydrate, pyrimidine, DNA and ornithine metabolism<sup>1-4</sup>. Recent investigations on purine metabolism showed that in the hepatomas there was an increased capacity in the *de novo* pathway of biosynthesis of inosine 5'-monophosphate (IMP), as reflected in the increased activity of glutamine PRPP amidotransferase (EC 2.4.2.14) and a decrease in IMP catabolism<sup>5,6</sup>. These observations directed our attention to the metabolic fate of IMP because this purine nucleotide is at a strategic position in purine metabolism (Fig. 1). Control at such branching points is exerted primarily by modification of the activity or of the rate of synthesis of the first enzymes of the divergent pathways. In this instance, in normal conditions, a balance is maintained by both positive and negative feedback effects. Thus ATP is required for GMP biosynthesis and GTP for AMP biosynthesis; conversely, GMP and AMP each inhibit their own production<sup>7</sup>. In normal or malignant proliferation, or in response to a hormone stimulus, changes in control frequently involve reprogramming of gene expression. The theoretical framework and predictive value of generalisations about such biochemical changes (termed the molecular correlation concept) have been elaborated<sup>1,3</sup>.

Briefly, in tumours, three classes of behaviour of enzymes and pathways were distinguished. Enzymes or pathways of the first group show a positive or negative correlation with tumour growth rate; these alterations seem to indicate a linking with the degree of expression of malignancy. Parameters of the second group are consistently increased or decreased in all tumours, regardless of growth rate, and thus seem to be linked with the malignant transformation *per se*. The third group includes parameters which show apparently random increases or decreases<sup>5</sup>. For a fuller understanding of the control of the guanine nucleotide pathway, therefore, it became necessary to study the first enzyme of this pathway, IMP dehydrogenase (IMP: NAD oxidoreductase, EC 1.2.1.14) in conditions of normal and neoplastic growth. This enzyme has been examined in several mammalian tissues<sup>8-16</sup>, including mouse sarcoma cells<sup>12</sup> and human placenta<sup>14</sup>. Comparative studies in homologous normal and malignant tissues have not, however, been reported.

IMP dehydrogenase cannot be measured accurately in crude tissue homogenates, because of interfering activities. The extraction technique and optical assay described in the legend to Fig. 2 were developed, from earlier methods<sup>10,12</sup>, to give optimal activity with liver and hepatoma preparations. In the conditions described, rates were linear for at least 30 min, and proportional to enzyme concentration up to a level of 0.0015 IU ml<sup>-1</sup>. No measurable nucleotidase activity was present. Normal and tumour-bearing



**Fig. 1** Simplified schematic metabolic map of the two principal competing routes for utilisation of inosine 5'-monophosphate. IMP, inosine 5'-monophosphate; SAMP, adenylosuccinate; XMP, xanthosine 5'-monophosphate; AMP and ATP, adenosine 5'-mono- and triphosphates; GMP and GTP, guanosine 5'-mono- and triphosphates, respectively.

rats were maintained as described previously and the preparation of regenerating liver, studies on differentiating animals, killing of rats and excision of livers and neoplasms were as reported elsewhere<sup>1-3</sup>. The growth rates of the various hepatoma lines ranged from 2 weeks to 11.5 months, measured by the time required for the tumours to reach a diameter of 1.5 cm. The IMP dehydrogenase activity and protein content in 11 transplantable hepatomas and in livers of control normal rats are compared in Fig. 2. In liver, the mean activity was 2,140 pmol substrate converted per h per mg of protein present in the 100,000g supernatant (coefficient of variation 15%). One gram (wet weight) of liver contained 88 mg of soluble protein. IMP dehydrogenase activity was elevated in all the hepatomas examined; even in the slowest growing tumours, with intervals of over a year between successive transplants, at least a 2.5-fold increase was seen. Moreover, the enzyme activity correlated with growth rate, and the increase, relative to normal liver, reached 13-fold in the most rapidly proliferating hepatomas. All increases were statistically significant at the 0.1% level, and the coefficient of correlation of IMP dehydrogenase activity with the exponential growth rate constant was 0.945 ( $P < 0.001$ ). The enzyme activity in renal tumour MK3 was increased threefold over the value observed in normal control kidney cortex.

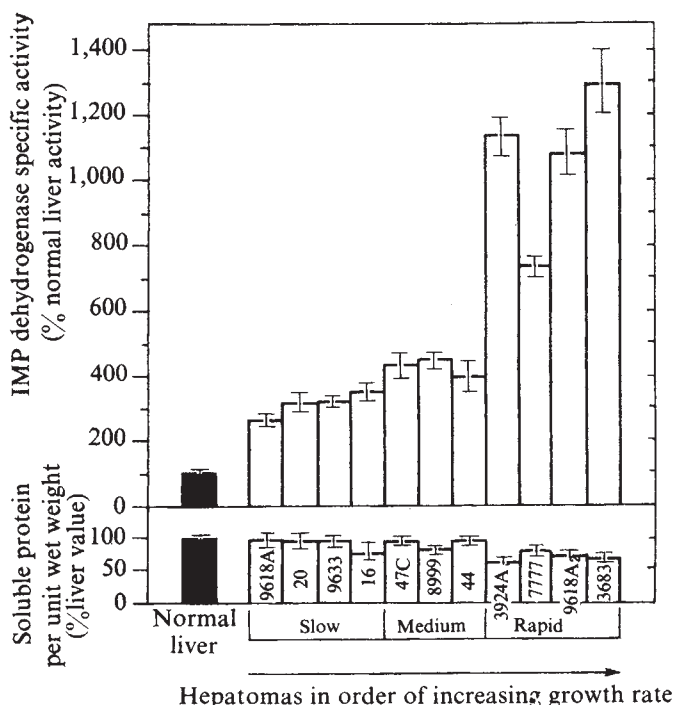
It was important to determine whether the IMP dehydrogenase of hepatomas had different properties from the liver enzyme. For this purpose the rapidly growing hepatoma 3924A was chosen. The pH profiles of the liver and hepatoma enzymes were identical, with optima at pH 8.1. Both enzymes were inhibited by excess  $\text{NAD}^+$  at concentrations above 0.25 mM, the inhibition reaching 50% at 0.62 mM. Both enzymes were also subject to product inhibition by XMP (competitive with IMP and non-competitive with  $\text{NAD}^+$ ) and by NADH (non-competitive with both IMP and  $\text{NAD}^+$ ). Liver and hepatoma dehydrogenases were inhibited equally by NADPH (50% inhibition, under usual assay conditions, at 0.14 mM) and by GMP (competitive with IMP,  $K_i$  96 mM). These patterns, together with initial velocity data, suggested that the kinetic mechanism of liver and hepatoma IMP dehydrogenases was identical with that of the sarcoma 180 and human placenta enzymes<sup>12,14</sup>. Within the limits of experimental error, kinetic parameters were identical for the liver and hepatoma enzymes, and were as follows (the terminology is that of Cleland<sup>17</sup>):  $K_a$  for IMP,  $11.6 \pm 2.5 \mu\text{M}$ ;  $K_{ia}$  for IMP,  $34.6 \pm 4.6 \mu\text{M}$ ;  $K_b$  for  $\text{NAD}^+$ ,  $24.3 \pm 3.8 \mu\text{M}$ ;  $K_i$  for XMP,  $25.9 \pm 3.1 \mu\text{M}$ ;  $K_i$  (slope) for NADH,  $360 \pm 66 \mu\text{M}$ ;  $K_i$  (intercept) for NADH,  $260 \pm 10 \mu\text{M}$ . Thus, the hepatoma

IMP dehydrogenase has properties similar to those of the normal liver enzyme.

To study the behaviour of IMP dehydrogenase in proliferating, non-malignant, adult hepatic tissue, we used the technique of partial hepatectomy<sup>18</sup>. Figure 3 shows that in the regenerating liver IMP dehydrogenase activity started to rise 6 h after operation, reached a peak of about five times the normal activity by 24 h, and then declined rather slowly. It is noteworthy how early the activity started to increase; most of the key enzymes of nucleic acid biosynthesis only start to rise after 12 h<sup>2,18</sup>. Another valuable system for the study of control of hepatic enzymes is the neonatal liver, which provides a population of differentiating, proliferating liver cells. On a basis of enzyme activity per unit of wet weight, the activity of IMP dehydrogenase in livers of 1-d-old rats is nearly four times the adult level, slowly declining thereafter with increasing age. But the average size of liver cells in newborn rats is less than 40% of the adult size. When our results were expressed on the basis of IMP dehydrogenase activity per cell (cell counts being determined by visual counting of orcein-stained nuclei in homogenates<sup>1</sup>) the per-cell activity in neonatal liver was only slightly (25%) higher than the adult value.

To evaluate this enzyme in the context of cell proliferation and function in the whole animal we measured IMP dehydrogenase in a wide variety of rat tissues. The highest activities were found in thymus, spleen, bone marrow and testis, which were, respectively, 10.5, 8.6, 3.3 and 2.1 times the liver specific activity. Successively lower activities were observed in brain, lung, intestinal mucosa, renal cortex, adipose tissue, red blood cells, and skeletal and cardiac muscle.

**Fig. 2** IMP dehydrogenase activity in rat hepatomas. 10% (w/v) homogenates were prepared in 0.25 M sucrose, buffered to pH 7.4 with Tris-chloride. 100,000g supernatants were fractionated with ammonium sulphate, and the fraction precipitating between 30% and 40% saturation contained all the IMP dehydrogenase activity. This precipitate was resuspended and dialysed against  $2 \times 200$  volumes of assay buffer (0.1 M potassium phosphate, pH 7.4 plus EDTA, 1 mM and mercaptoethanol, 0.5 mM). Samples were assayed at 290 nm, in the same buffer, at 37 °C in a Gilford 2400-S recording spectrophotometer. The concentration of  $\text{NAD}^+$  in the assay was 0.2 mM and that of IMP was 0.17 mM. The protein concentration of the 100,000g supernates was determined by the Biuret reaction. The data are means  $\pm$  s.e. of five or more animals in each group and results are plotted as percentages of corresponding control liver values.





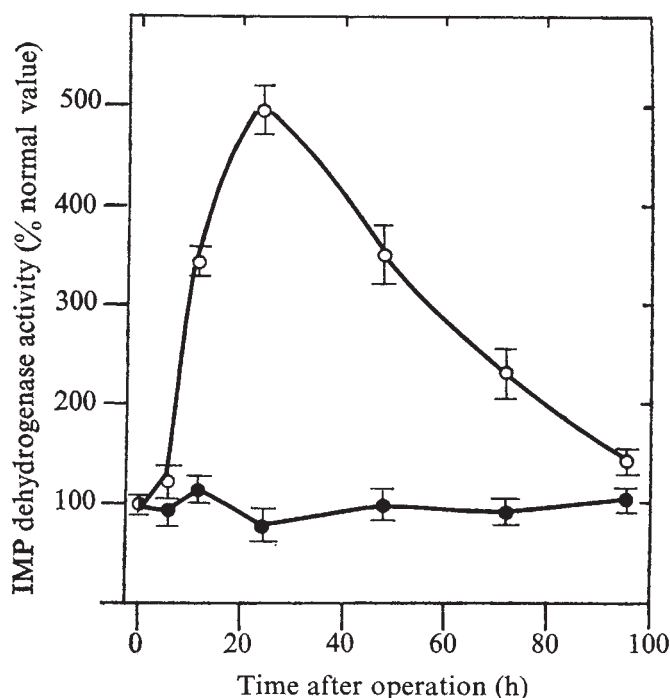


Fig. 3 IMP dehydrogenase in rat liver following partial hepatectomy (○) and sham operation (●). Enzyme was prepared and assayed as described in Fig. 2.

From this study a number of conclusions may be drawn. First, a relationship of IMP dehydrogenase activity with cell proliferation is evident. This correlation is most clearly apparent in the spectrum of transplantable hepatomas of different growth rates, and is further emphasised by the high activity found in regenerating liver, and in thymus and spleen. The correlation, however, is not absolute, as the lower activities in neonatal liver and in intestinal mucosa demonstrate. Second, the low absolute activity of this enzyme, even in the most rapidly dividing tissues, suggests that it may be one of the key rate-controlling enzymes in nucleic acid biosynthesis. The activity found in regenerating liver, for example, would be sufficient to allow the liver RNA and DNA content to be doubled in about 18 h. In fact, the doubling time for regenerating liver is about 4 d; it seems that under these conditions, and also in the fastest-growing tumours, IMP dehydrogenase must operate at around 20% of its maximum velocity. Last, the regulation of the synthesis of this enzyme is undoubtedly changed in malignancy. Even the slowest growing hepatomas with mass-doubling times of more than 40 d, have a 2.5 to 3.5-fold elevation compared with normal liver, and the rapid hepatomas, with growth rates comparable with that of regenerating liver<sup>1,3</sup>, have, on average, almost twice as much IMP dehydrogenase activity as is observed at the peak following partial hepatectomy. Moreover, in hepatomas, the enzyme activity elevation is permanent.

The ubiquitous increase of IMP dehydrogenase in hepatomas indicates the linking of this enzyme alteration with the neoplastic transformation. Superimposed on this reprogramming of gene expression is the positive correlation of this enzyme activity with the rate of growth of the liver neoplasms, indicating a linking of this enzymatic imbalance also with the expression of the degrees of malignancy.

This work was supported by grants from the US Public Health Service.

ROBERT C. JACKSON  
GEORGE WEBER

Laboratory for Experimental Oncology and  
Department of Pharmacology,  
Indiana University School of Medicine,  
Indianapolis, Indiana 46202

HAROLD P. MORRIS

Department of Biochemistry,  
Howard University College of Medicine,  
Washington DC 20001

Received April 28; accepted May 22, 1975.

- Weber, G., in *The Molecular Biology of Cancer* (edit. by Busch, H.), 487–521 (Academic, New York, 1974).
- Weber, G., Queener, S. F., and Ferdinandus, J. A., *Adv. Enzyme Regul.*, **9**, 63–95 (1971).
- Weber, G., *Adv. Enzyme Regul.*, **11**, 79–102 (1973).
- Williams, J. C., Weber, G., and Morris, H. P., *Nature*, **253**, 567–569 (1975).
- Katunuma, N., and Weber, G., *FEBS Lett.*, **49**, 53–56 (1974).
- Weber, G., Prajda, N., and Williams, J. C., *Adv. Enzyme Regul.*, **13**, 3–25 (1975).
- Hartman, S. C., in *Metabolic Pathways*, third ed., 4 (edit. by Greenberg, D. M.), 1–69 (Academic, New York, 1970).
- Abrams, R., and Bentley, M., *Archs Biochem.*, **56**, 184–195 (1955).
- Atkinson, M. R., Morton, R. K., and Murray, A. W., *Biochem. J.*, **89**, 167–172 (1963).
- Al-Mudhaffar, S., and Ackerman, C. J., *Endocrinology*, **82**, 912–918 (1968).
- Pehlke, D. M., McDonald, J. A., Holmes, E. W., and Kelley, W. N., *J. clin. Invest.*, **51**, 1398–1404 (1972).
- Anderson, J. A., and Sartorelli, A. C., *J. biol. Chem.*, **243**, 4762–4768 (1968).
- McFall, E., and Magasanik, B., *J. biol. Chem.*, **235**, 2103–2108 (1969).
- Holmes, E. W., Pehlke, D. M., and Kelley, W. N., *Biochim. biophys. Acta*, **364**, 209–217 (1974).
- Saccoccia, P. A., and Miesch, R. P., *Molec. Pharmacol.*, **5**, 26–29 (1969).
- Sweeney, M. J., Hoffman, D. H., and Esterman, M. A., *Cancer Res.*, **32**, 1803–1809 (1972).
- Cleland, W. W., *Biochim. biophys. Acta*, **67**, 104–137 (1963).
- Bucher, N. L. R., and Malt, R. A., *Regeneration of Liver and Kidney* (Little, Brown, Boston, 1971).

## Efficiency of light conversion by the blue-green alga *Anacystis nidulans*

THE conversion efficiency of light energy into chemical energy, measured by production of organic matter, can be high with unicellular organisms. As photosynthesis becomes light saturated at relatively low light intensities, such high efficiencies are not usually obtained with high intensities of incident light. The saturation level, however, increases with increasing temperature. Blue-green algae grow well at high temperatures<sup>1–3</sup>, suggesting that they may be efficient producers of organic matter. As the protein content of algae in general is high, growth of these organisms could have important implications for food production, especially in tropical areas, where both light intensity and temperature are favourable. We have therefore investigated some parameters influencing yield of production of *Anacystis nidulans* at high light intensities.

High growth rates require an adequate supply of carbon dioxide for cell production. As a result of a high ratio of outer surface area chlorophyll content, favourable for CO<sub>2</sub> diffusion, unicellular organisms can profit much more from enhanced CO<sub>2</sub> availability than leaves of higher plants.

Using a medium according to Kratz and Myers<sup>4</sup> we found that an increase in A<sub>680</sub> of 42% (the *in vivo* chlorophyll maximum) resulted from 6 h illumination with incandescent lamps (50 W m<sup>-2</sup> photosynthetically active light), if the cells were flushed with air enriched with 5% CO<sub>2</sub>. The absorbance increase for a culture flushed with plain air was only 10% of that of cells flushed with enriched air, indicating that CO<sub>2</sub> was a limiting factor in these conditions. In the latter culture the phycocyanin–chlorophyll ratio, measured by A<sub>625</sub> and A<sub>680</sub>, decreased from a value of 1.4 to 1.8, found with rapidly growing cells, to about 0.8 (Fig. 1). A marked increase in growth rate and in phycocyanin–chlorophyll ratio resulted if these cultures were switched from air to CO<sub>2</sub>-enriched air. Such an increase also followed when the rate of flushing with air was tenfold increased and the bubble size decreased. If the culture was flushed with plain air but 2% sodium bicarbonate was added to the culture medium and the pH readjusted to 7.5, growth proceeded at a rate similar to that of cells flushed by CO<sub>2</sub>-enriched air. After 1.5 d, however, the pH of this culture had risen to 11, phycocyanin was bleached and the cells eventually died.

An intensity of 50 W m<sup>-2</sup> from incandescent lamps was not sufficient for maximal growth with cells flushed with CO<sub>2</sub>-enriched air, indicating that photosynthesis was not

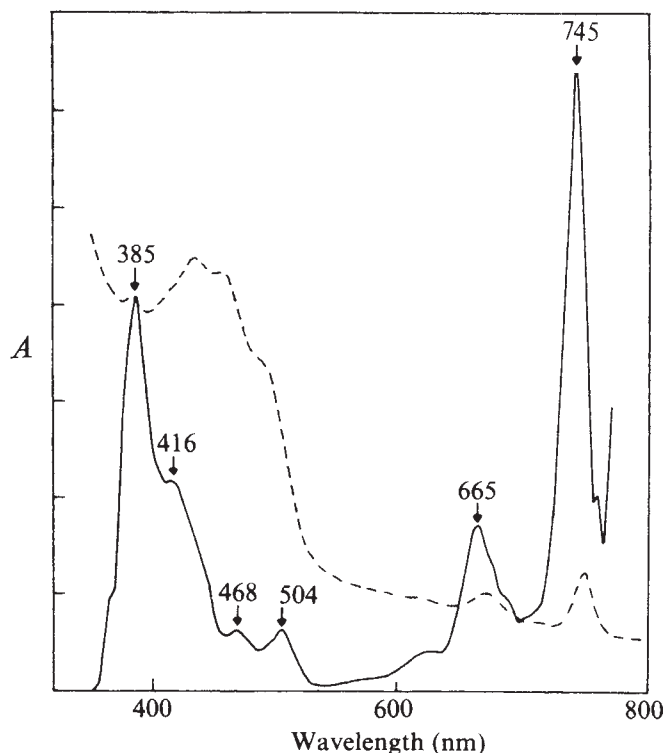
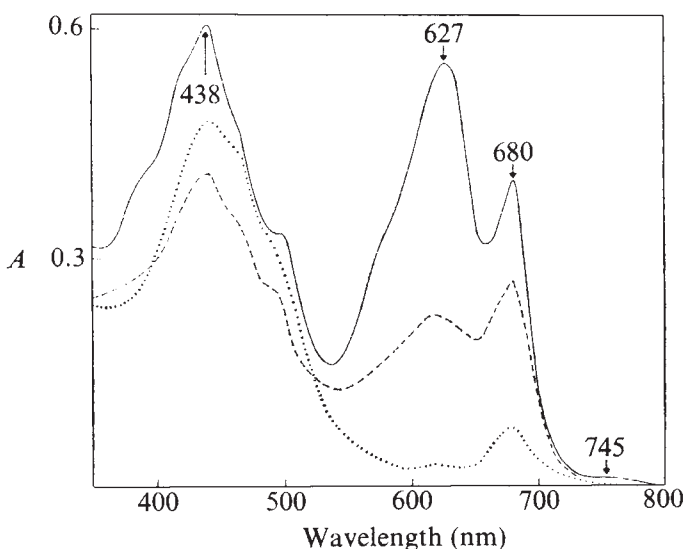
saturated. If the intensity was doubled, the rate of absorption increase also doubled (temperature 40 °C, flushing rate 8 l h<sup>-1</sup>).

After 4 d continuous illumination with 50 W m<sup>-2</sup>, growth stopped. Removal of the cells by centrifugation and renewed inoculation with fresh cells in the same phase of growth as at the start of the experiment did not result in resumed growth. Investigation of the ultraviolet absorption spectrum of the supernatant suggested that at least the nitrogen source was depleted. Removal of the cells, renewed inoculation and addition of 50% fresh growth medium resulted in a vigorous growth for 18 h. After that time the cells turned yellow and growth stopped. The absorption spectrum of the yellow cells and absorption difference spectra with normal cells indicated that phycocyanin disappears, chlorophyll concentration decreases and that of a carotenoid increases markedly (Fig. 1). Thin-layer chromatography of acetone extracts from the cells showed that the same acetone-soluble pigments are present in both types of cell, but the yellow ones contain, besides less chlorophyll, an appreciably greater content of a xanthophyll. Ultraviolet absorption spectra of the supernatant indicated the excretion of various polypeptides<sup>5</sup>, which may act as growth inhibitors and modifiers of the absorption spectrum.

*Anacystis* receiving light from fluorescent tubes (Philips TL 33, also equivalent to 50 W m<sup>-2</sup>) grow at a similar rate to cells illuminated with incandescent lamps in the same conditions of CO<sub>2</sub> availability, temperature and light intensity. Cells irradiated with white light from a high pressure mercury lamp (from which the ultraviolet was eliminated) at the same energy, grow about eight times less rapidly. Besides an inefficient absorption ratio by the two photosynthetic pigment systems, the latter effect may be the result of the large fraction of blue light absorbed by xanthophylls. No energy transfer from these pigments to chlorophyll of either pigment system is found in blue-green algae<sup>6</sup>.

We also found that a pigment system, containing both xanthophylls and the 745 nm pigment which occurs in *Anacystis*<sup>7,8</sup> could be precipitated from lamellae treated with sodium dodecyl sulphate (SDS). The fluorescence action spectrum of the 753 nm fluorescence band, emitted by the latter pigment form, shows bands in the carotenoid region, indicating some energy transfer between these non-

**Fig. 1** Absorption spectrum of *Anacystis* grown for 2 d in a medium flushed with air + 5% CO<sub>2</sub> (....) and plain air (---, × 12) at 35 °C and 1.2 10<sup>4</sup> lx from incandescent lamps. Continuous curve gives the absorption spectrum of yellow cells, obtained after reinoculation of exhausted growth medium with fresh cells and addition of 50% fresh medium.



**Fig. 2** Fluorescence action spectrum and absorption spectrum of a pigment complex isolated by SDS treatment from *Anacystis* cells which were disintegrated by the French press method. The complex emits a fluorescence band with a maximum at 753 nm. ---, A; —, fluorescence excitation.

fluorescent xanthophylls and fluorescing P745 (Fig. 2). The function of this pigment system in the energy scheme of this alga is not yet understood.

The growth pattern in the first few days after inoculation shows that, under favourable conditions of CO<sub>2</sub> availability, nutrients, temperature and spectral composition of the light, photosynthesis proceeds with much greater efficiency, even at high light intensities, than in the green alga *Chlorella*<sup>9</sup>.

From the values measured during growth the conversion yield *Q* of electromagnetic into chemical energy can be calculated. An irradiation intensity of 50 W m<sup>-2</sup> on a bottle with 200 cm<sup>2</sup> side area (volume 1 l) corresponds, after 1.5 d growth, to 3.6 kcalorie radiant energy taken up by the cells in 6 h (average *A* 700–400 75%). This illumination results in a cell production with a dry weight of 0.20 g l<sup>-1</sup>. According to Oswald<sup>10</sup> the energy content of algae in general varies between 4.6 and 6.5 kcalorie g<sup>-1</sup>, a value close to that derived from Rabinowitch<sup>11</sup>. If we take an average value of 5.5 kcalorie g<sup>-1</sup>, *Anacystis* cells produced in 6 h, contain an energy equivalent to 1.1 kcalorie. From these values we obtain a conversion factor (energy efficiency) of *Q* = 0.30. The latter is only an approximate value constant over most of the growth period and is of the same order of magnitude as that expected from the optimal values for photosynthesis measurements, corresponding to a quantum efficiency of eight and derived from short time experiments<sup>11</sup>.

Our measurements indicate that *Anacystis* may be grown in conditions in which high intensity radiation is converted into chemical energy with a high yield.

J. C. GOEDHEER

J. W. KLEINEN HAMMANS

Biophysics Research Group,  
State University, Utrecht, The Netherlands

Received April 4; accepted June 2, 1975.

<sup>1</sup> Haldall, P., *Physiologia Pl.*, **11**, 401–420 (1958).



- <sup>2</sup> Carr, N. G., and Whitton, B. A., *The Biology of Blue-Green Algae*, 360–367 (Blackwell, Oxford, 1973).  
<sup>3</sup> Fogg, G. E., Stewart, W. D. P., Fay, P., and Walsby, A. E., *The Blue-green Algae*, 129–142 (Academic, London, 1973).  
<sup>4</sup> Kratz, W., and Myers, J., *Am. J. Bot.*, **42**, 282–287, (1955).  
<sup>5</sup> Fogg, G. E., *Proc. R. Soc.*, **B139**, 372–397 (1952).  
<sup>6</sup> Goedheer, J. C., *Biochim. biophys. Acta*, **172**, 252–265 (1969).  
<sup>7</sup> Gassner, E. B., *Pl. Physiol. Lancaster*, **37**, 637–639 (1962).  
<sup>8</sup> Fischer, K., and Metzner, H., in *Progr. Photosynth. Res.*, **2**, (edit. by Metzner, H.), 547–551 (Tübingen, 1969).  
<sup>9</sup> Wassink, E. C., Kok, B., and Van Oorschot, J. L. P., in *Algal Culture, from Laboratory to Pilot Plants*, (edit. by Burlew, J. S.), 55–62 (Carn. Inst. Wash. Publ. no. 600, Washington, DC, 1953).  
<sup>10</sup> Oswald, W. J., in *Proc. Workshop Bio-Solar Conversion*, **38**, (NSF/RANN report, 1973).  
<sup>11</sup> Rabinowitch, E., *Photosynthesis and Related Processes*, **1**, 49 and **2**, 1946 (Wiley, New York, 1945 and 1956).

## Evidence for heterogeneity in heterochromatin of *Drosophila melanogaster*

IN *Drosophila melanogaster* the heterochromatin comprises the whole of the Y chromosome, about the proximal third of the X chromosome and the centromeric areas of chromosomes 2 and 3<sup>1–4</sup>. It appears throughout the cell cycle as darkly staining and highly condensed chromatin<sup>2,3</sup> and replicates later than euchromatin during the synthetic period<sup>5</sup>. The heterochromatin of *Drosophila* is considered genetically inert because it contains very few mappable genes, although it has marked genetic effects in determining the well known position effect<sup>1</sup>. It has recently been found that the heterochromatin of *Drosophila* corresponds to the C bands<sup>6</sup> and contains highly repetitive DNA<sup>7–12</sup>. None of these characteristics has, however, so far been of any use in solving the problem of the functional role of the heterochromatin. On the other hand, *Drosophila* offers unique possibilities for solving this problem, since in this organism it is possible to construct chromosomes containing different quantities of centromeric heterochromatin<sup>4,13</sup> and thus study the functions of this material. Obviously this type of approach requires a preliminary study on the possible heterogeneity of the heterochromatin. An initial result in this type of study has been obtained by showing that the heterochromatin regions of *D. melanogaster* fluoresce differently after staining both with quinacrine<sup>14–16</sup> and with the compound 33258 Hoechst<sup>17</sup>. Here we describe experiments in which the heterochromatin of *Drosophila* was differentiated by means of treatment of the living ganglionic cells with 33258 Hoechst, which is known to decondense the centric heterochromatin of the mouse<sup>18</sup>.

Use was made of third instar larvae of the Oregon stock and of a stock heterozygous for the translocation (2;3) bw<sup>4</sup> (ref. 4). The nerve ganglia obtained from the dissection of the larvae were placed in a water solution containing NaCl (0.7%), foetal calf serum (20%) and 33258 Hoechst (40 µg ml<sup>-1</sup>). After 2, 4, 6 and 8 h in this solution the ganglia were fixed and then squashed in acetic orcein<sup>19,20</sup>; 2 h before each fixation, colchicine was added to the culture solution, up to a final concentration of 10<sup>-4</sup> M.

Figure 1 shows the effects of the treatment of the living ganglionic cells with 33258 Hoechst. As may be seen, this treatment produces no alteration in the euchromatin, whereas it has a very marked effect on certain heterochromatic regions in which specific areas of decondensation are clearly visible. We emphasise that we observed no metaphases with chromosome aberrations or with heterochromatin decondensation patterns different from those shown in Fig. 1. The frequency of the various types of metaphase shown in Fig. 1 varies, however, with the fixation time (Table 1).

The data shown in Fig. 1 and Table 1 reveal that the heterochromatin of *D. melanogaster* responds in the following way to the treatment with 33258 Hoechst.

(1) The heterochromatin of the second chromosomes is not influenced.

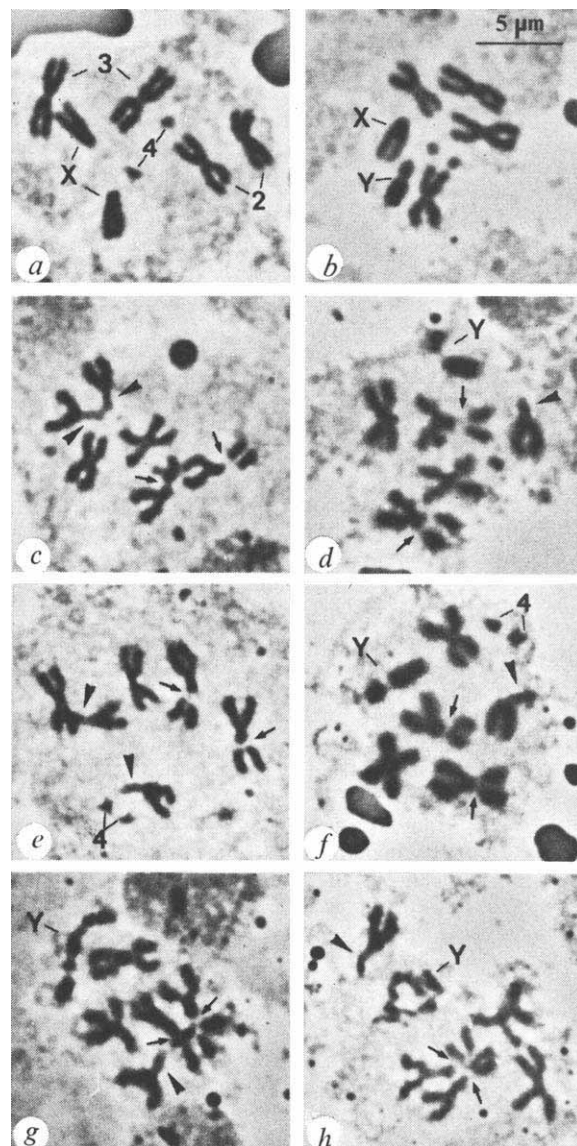


Fig. 1 The effect of 33258 Hoechst on the metaphase chromosomes of ganglionic cells of *Drosophila melanogaster*: a, ♀ and b, ♂ normal non-treated cells; c, ♀ and d, ♂ cells treated for 4 h with H33258; e, ♀ and f, ♂ cells treated for 6 h with H33258; g, ♂ and h, ♂ cells treated for 8 h with H33258. In all the treated cells an area of drastic decondensation of the centromeric heterochromatin of a single arm of the 3rd chromosomes (↑) and the intermediate decondensation of part of the heterochromatin of the X chromosome (▲) can be seen. The male cells (d) and (f) show an area of drastic decondensation close to the centromere of the Y chromosome. In the male cells (g) and (h) the Y chromosome appears intermediately decondensed with the exception of some blocks of heterochromatin that remain compact. In the metaphases (e) and (f) the 4th chromosomes appear elongated and with the sister chromatids partially split. As may be seen the regions of drastic decondensation on the third chromosomes and on the Y chromosome seem to be isochromatid breaks. Under the microscope, however, it is often possible to observe very slender filaments of chromatin that connect the apparently broken chromatid ends.

(2) The heterochromatin of the right arm of the third chromosomes is not influenced. On the other hand, a distal portion with respect to the centromere of the heterochromatin of the left arm is drastically decondensed. The degree of decondensation of this heterochromatin portion does not vary with the time of fixation. The area of decondensation was assigned to the left arm of the third chromosome by using the stock heterozygous for the translocation (2;3) bw<sup>4</sup> (Fig. 2).

**Table 1** Effects of 33258 Hoechst on ganglial cells of *D. melanogaster* at various times of fixation after the treatment

| Post-treatment<br>time of fixation<br>(h) | Sex | No. of cells<br>scored | Normal<br>cells (%) | Cells showing (%) |                          |                       |                                |                                |
|-------------------------------------------|-----|------------------------|---------------------|-------------------|--------------------------|-----------------------|--------------------------------|--------------------------------|
|                                           |     |                        |                     | d.d.3<br>i.d.X    | d.d.3<br>i.d.X<br>d.d.Y. | d.d.3<br>i.d.X<br>e.4 | d.d.3<br>i.d.X<br>d.d.Y<br>e.4 | d.d.3<br>i.d.X<br>t.d.Y<br>e.4 |
|                                           |     |                        | (Fig. 1a, b)        | (Fig. 1c)         | (Fig. 1d)                | (Fig. 1e)             | (Fig. 1f)                      | (Fig. 1g, h)                   |
| 2                                         | ♀♀  | 484                    | 79.2                | 20.8              | —                        | —                     | —                              | —                              |
|                                           | ♂♂  | 588                    | 83.7                | —                 | 16.3                     | —                     | —                              | —                              |
| 4                                         | ♀♀  | 179                    | 48.0                | 52.0              | —                        | —                     | —                              | —                              |
|                                           | ♂♂  | 616                    | 49.8                | —                 | 50.2                     | —                     | —                              | —                              |
| 6                                         | ♀♀  | 132                    | 10.7                | 63.1              | —                        | 26.2                  | —                              | —                              |
|                                           | ♂♂  | 386                    | 13.2                | —                 | 71.5                     | —                     | 1.2                            | 14.1                           |
| 8                                         | ♀♀  | 153                    | —                   | 20.8              | —                        | 79.2                  | —                              | —                              |
|                                           | ♂♂  | 132                    | —                   | —                 | 12.6                     | —                     | 2.3                            | 85.1                           |

Abbreviations: d.d.3=drastic decondensation on the third chromosomes; i.d.X=intermediate decondensation on the X chromosome; d.d.Y=drastic decondensation on the Y chromosome; e.4=fourth chromosomes elongated; t.d.Y.=total decondensation, except for certain regions, of the Y chromosome.

(3) Part of the heterochromatin of the X chromosome (presumably the greater part of that localised in XL) is not influenced; the remaining part, at all times of fixation, is seen to be intermediately decondensed.

(4) The Y chromosome, at all times of fixation, displays an area of drastic decondensation near the centromere. After 6–8 h of treatment it all appears intermediately decondensed with the exception of some blocks of heterochromatin which remain compact.

It is interesting to note that in the cells treated for 6–8 h the fourth chromosomes also appear elongated and with the sister chromatids split. We consider that this results from decondensation of heterochromatic material, since the fourth chromosomes, although they show no positive heteropycnosis in interphase<sup>2,3</sup>, nevertheless display heterochromatic genetic properties<sup>4</sup>, are late replicating<sup>5</sup> and positive in the C bands<sup>6</sup>.

Taken as a whole, the present data indicate that, in relation to treatment with 33258 Hoechst, the heterochromatin of *D. melanogaster* can be divided into at least

three types: that which is insensitive to this compound, that which is intermediately decondensed and that which is drastically decondensed. It is, however, possible that there are differences between the heterochromatin of the X chromosome, which decondenses right from the first fixation times, and that of the Y chromosome and the fourth chromosomes, which decondense after 6–8 h of treatment.

As regards the nature of the areas sensitive to the treatment with Hoechst, we believe that, in all probability, they contain DNA that is rich in AT. This is suggested by two considerations. First, the compound 33258 Hoechst decondenses all the mouse heterochromatic centric regions<sup>18</sup> in which we know that satellite DNA rich in AT is localised<sup>21,22</sup>. Second, all heterochromatic areas of the chromosomes of *Drosophila*, decondensed by the bibenzimidazole derivative correspond to areas that fluoresce brightly after staining with quinacrine<sup>14–16</sup> or 33258 Hoechst<sup>17</sup>, which are both fluorescent probes specific for DNA rich in AT<sup>17,23–25</sup>.

It is more difficult to establish the respective nature of the intermediately decondensed and the drastically decondensed regions. In principle, however, it is logical to assume that the degree of decondensation depends on the relative AT concentration in these regions. In this respect it could be suggested that in the intermediately decondensed regions are localised the two light satellites of *D. melanogaster*, which band at an approximate density of 1.690 g cm<sup>-3</sup> in neutral CsCl (refs 8 and 10). The drastically decondensed regions, on the other hand, might contain the *Drosophila* d(A–T) rich satellite DNA<sup>10,26,27</sup> (1.675 (ref 26)—1.678 (ref 27)—1.672 (ref 10) g cm<sup>-3</sup>). In this respect it is interesting to note that all these light satellites have been shown, by *in situ* hybridisation, to have chromocentric localisation in the polytene chromosome preparations<sup>8,10</sup>. Furthermore the d(A–T) rich satellite DNA would be localised preferentially, though not exclusively, in the Y chromosome<sup>26</sup>, which, as we have shown, contains a region of drastic decondensation.

We thank Professor G. Olivieri for a critical reading of the manuscript, and Miss M. P. Belloni for technical assistance. Further thanks are due to Dr H. Loewe for providing samples of 33258 Hoechst.

S. PIMPINELLI  
M. GATTI  
A. DE MARCO

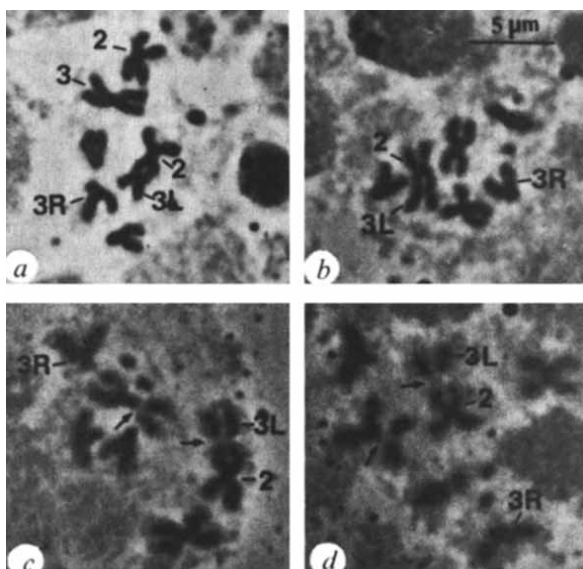
Centro di Genetica Evolutiva del CNR,  
Istituto di Genetica,  
Università di Roma,  
Rome, Italy

Received April 3; accepted May 28, 1975.

<sup>1</sup> Hannah, A., *Adv. Genet.* 4, 87–121 (1951).

<sup>2</sup> Heitz, E., *Z. Zellforsch.* 20, 237–287 (1933).

**Fig. 2** Localisation on the left arm of the third chromosome of the region of drastic decondensation induced by 33258 Hoechst: nerve ganglia of larvae heterozygous for the translocation (2; 3) bw<sup>4</sup> (points of breakage in 2R near bw and in 3L near centromere<sup>4</sup>) were fixed after 4h of treatment with H33258 (40 µg ml<sup>-1</sup>). In the treated cells it may be clearly observed that the 3L arm translocated on to 2R shows the heterochromatic area drastically decondensed; however, the 3R arm is not influenced. *a*, ♀ and *b*, ♂ untreated cells heterozygous for t(2; 3) bw<sup>4</sup>; *c*, and *d*, ♂ the same type of cells fixed after 4 h of treatment with 33258 Hoechst.





- <sup>3</sup> Kaufmann, B. P., *J. Morph.*, **56**, 125–155 (1934).  
<sup>4</sup> Lindsley, D., and Grell, E. H., *Carnegie Inst. Wash. Publ. No. 627* (1968).  
<sup>5</sup> Barigozzi, C., Dolfini, S., Fraccaro, M., Rezzonico-Raimondi, G., and Tiepolo, L., *Expl Cell Res.*, **43**, 231–234 (1966).  
<sup>6</sup> Hsu, T. C., *J. Hered.*, **62**, 285–287 (1971).  
<sup>7</sup> Botchan, M., Kram, R., Schmid, C. W., and Hearst, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1125–1129 (1971).  
<sup>8</sup> Gall, J. G., Cohen, F. H., and Polan, M. D., *Chromosoma*, **33**, 319–344 (1971).  
<sup>9</sup> Jones, K. W., and Robertson, F. W., *Chromosoma*, **31**, 331–345 (1970).  
<sup>10</sup> Peacock, W. J., et al., *Cold Spring Harb. Symp. quant. Biol.*, **38**, 405–416 (1973).  
<sup>11</sup> Perreault, W. J., Kaufman, B. P., and Gay, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 773–777 (1973).  
<sup>12</sup> Rae, P., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1018–1025 (1970).  
<sup>13</sup> Lindsley, D. L., et al., *Genetics*, **71**, 157–184 (1972).  
<sup>14</sup> Addison, K. P., Perreault, W. J., and Gay, H., *Chromosoma*, **34**, 190–205 (1971).  
<sup>15</sup> Faccio Dolfini, S., *Chromosoma*, **44**, 383–391 (1974).  
<sup>16</sup> Vosa, C. G., *Chromosoma*, **31**, 446–451 (1970).  
<sup>17</sup> Holmquist, G., *Chromosoma*, **49**, 333–356 (1975).  
<sup>18</sup> Hilwig, I., and Gropp, A., *Expl Cell Res.*, **81**, 474–477 (1973).  
<sup>19</sup> Gatti, M., Tanzarella, C., and Olivieri, G., *Nature*, **247**, 151–152 (1974).  
<sup>20</sup> Gatti, M., Tanzarella, C., and Olivieri, G., *Genetics*, **77**, 701–719 (1974).  
<sup>21</sup> Jones, K., *Nature*, **225**, 912–915 (1970).  
<sup>22</sup> Pardue, M. L., and Gall, J. G., *Science*, **168**, 1356–1358 (1970).  
<sup>23</sup> Ellison, J. R., and Barr, H. J., *Chromosoma*, **36**, 375–390 (1972).  
<sup>24</sup> Weisblum, B., and de Haseth, P. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 629–632 (1972).  
<sup>25</sup> Weisblum, B., and Haensler, E., *Chromosoma*, **46**, 255–260 (1974).  
<sup>26</sup> Blumenfeld, M., and Forrest, H. S., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3145–3149 (1971).  
<sup>27</sup> Fansler, B. S., Travaglini, E. C., Loeb, L. A., and Schultz, J., *Biochem. biophys. Res. Commun.*, **40**, 1266–1272 (1970).

## Poly(U) polymerase in rat brain

MUCH interest has centred recently on the discoveries of ribohomopolymer sequences in RNAs of eukaryotic cells. Poly(A)<sup>1–3</sup>, poly(U)<sup>4</sup> and poly(G)<sup>5</sup> regions have been found in nuclear and cytoplasmic RNA fractions. These sequences are thought to have special implications for regulation of biological functions of RNAs. It seems likely that these ribohomopolymer sequences are synthesised in a post-transcriptional process. Poly(A) polymerases have been reported<sup>6–7,13</sup> and enzymatic activities catalysing short (two to five nucleotides) chains of poly(U) have been reported in cytoplasmic and microsomal fractions in rat liver<sup>8,9</sup>. Enzymatic activities which polymerise four different ribohomopolymers independently have been found in nuclear ribonucleoprotein particles<sup>10</sup>.

We have reported the presence of RNA-dependent RNA polymerase requiring four nucleoside triphosphates as substrate in an enzyme fraction of rat brain<sup>11</sup>. With this in mind, a poly(U) polymerase was separated from the same organ and was shown to have a primer requirement for RNAs of larger size, such as ribosomal RNAs (rRNAs), and viral RNA. The enzyme catalyses sequential polymerisation of poly(U) chains from the 3'-hydroxyl end of RNA. We report here studies on the isolation and characterisation of a new poly(U) polymerase. The activity of poly(U) polymerase was demonstrated in a supernatant which was solubilised with 0.4 M ammonium sulphate from the microsomal fraction (see legend to Table 1). This enzyme activity had an absolute requirement for a primer. The presence of creatine phosphate and creatine kinase stimulated the reaction, indicating that ribonucleoside phosphorylase is not involved and that ribonucleoside triphosphates rather than diphosphates are the actual substrates. Only slight incorporations, less than 10% of incorporation of <sup>3</sup>H-UMP, were demonstrated in the presence of other nucleoside triphosphates (<sup>3</sup>H-ATP, <sup>3</sup>H-GTP and <sup>3</sup>H-CTP) as substrates (data not shown).

Primer specificity of the poly(U) polymerase was surveyed in the presence of several other primers (Table 2). rRNAs from rat brain, liver and *Escherichia coli* had higher activities than smaller RNAs in a transfer RNA (tRNA) fraction from the same sources. Kinetics of the synthesis showed that the efficiency of smaller RNA in a tRNA fraction as a primer was about 30% that of rRNA at any incubation time (Fig. 1). Q $\beta$  phage RNA also had a similar level of primer activity as rRNAs; but the primer activity of the fragmented Q $\beta$  phage RNA (4S) was the same as the activity of smaller RNAs in tRNA fraction. These results show the poly(U) polymerase preferred larger RNAs as a primer than smaller RNAs. The molecular

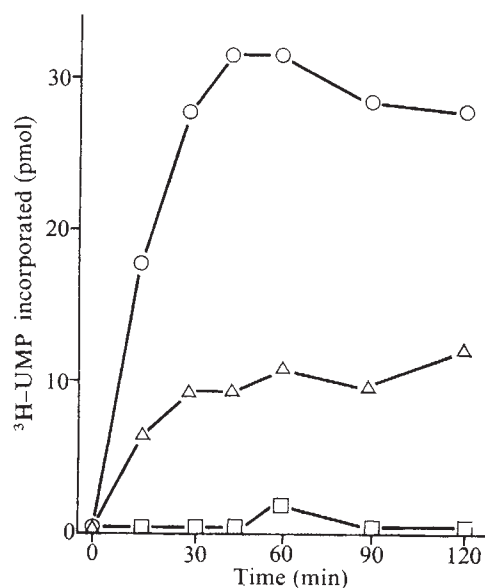
**Table 1** Properties of poly(U) polymerase from rat brain

| Assay conditions                                                      | <sup>3</sup> H-UMP incorporated (pmol) |
|-----------------------------------------------------------------------|----------------------------------------|
| Complete system                                                       | 26.8                                   |
| + DNase (10 $\mu$ g)                                                  | 27.9                                   |
| + RNase A (10 $\mu$ g)                                                | 0                                      |
| + Actinomycin D (2 $\mu$ g)                                           | 22.9                                   |
| – Mg <sup>2+</sup>                                                    | 0.9                                    |
| – Dithiothreitol                                                      | 18.7                                   |
| + ATP, GTP, CTP (0.1 $\mu$ mol each)                                  | 27.0                                   |
| + Phosphocreatine (1 $\mu$ mol) + creatine phosphokinase (10 $\mu$ g) | 34.9                                   |
| – RNA                                                                 | 0                                      |

Reaction mixture contained in a final volume of 0.25 ml: 22  $\mu$ mol Tris-HCl pH 8.3, 0.75  $\mu$ mol MgCl<sub>2</sub>, 0.125  $\mu$ mol dithiothreitol, 0.1  $\mu$ mol <sup>3</sup>H-UTP (2  $\times$  10<sup>6</sup> c.p.m. 0.1  $\mu$ mol<sup>-1</sup>), 5  $\mu$ g 28S rRNA from newborn rat brain, 20  $\mu$ g enzyme protein. Incubated at 36 °C for 30 min, stopped by addition of saturated pyrophosphate-sodium phosphate (1M), pH 7.4 (1:1) mixture and TCA. Enzymatic activity was measured by converting <sup>3</sup>H-labelled nucleoside monophosphates into acid-insoluble material. <sup>3</sup>H-labelled samples were counted in a liquid scintillation counter (Beckman LS 100). All procedures were done at 0 °C unless otherwise indicated. Wistar rats 8–12-weeks old were used. Fresh rat brain was homogenised in a glass-Teflon homogeniser with 10 volumes ice-cold buffer (10 mM Tris-HCl, pH 7.4, 15 mM KCl, 5 mM 2-mercaptoethanol). Mitochondria and cell debris were removed by centrifugation at 12,000g for 30 min. Supernatant was centrifuged at 105,000g for 60 min to obtain microsomal fraction. Microsomal pellet was suspended in 0.4 M ammonium sulphate solution, pH 7.4, and gently homogenised in a glass-Teflon homogeniser. After 60 min standing, microsomal fraction was centrifuged at 105,000g for 60 min. Supernatant was brought to 30% saturation by adding 100% saturated ammonium sulphate and left standing for 20 min. Resulting precipitate was removed by centrifugation at 17,000g for 10 min. The 30% saturated ammonium sulphate supernatant was brought to 45% saturation with solid ammonium sulphate and left standing for 20 min. Precipitate was collected by centrifugation at 17,000g for 10 min. Pellet was dissolved in SB (10 mM Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>, 0.5 mM 2-mercaptoethanol). Protamin sulphate (30  $\mu$ g ml<sup>-1</sup>) was added to the dissolved pellet and was centrifuged at 17,000g for 10 min after 20 min of standing. An equal volume of 100% saturated ammonium sulphate was added to the supernatant, and after 20 min standing, pellet was collected by centrifugation at 17,000g for 10 min. Precipitate was dissolved in SB and 0.3 volumes glycerol was added to the medium. This enzyme fraction was stored at –60 °C.

weight of the tRNA is known to be about 20,000, and that of Q $\beta$  RNA 1,000,000. Considering the molecular sizes of tRNA and rRNA, tRNA should show higher activity at the same absorbance level, and this may also be true for

**Fig. 1** Time course of reaction of poly(U) polymerase. Conditions of incubations were as in Table 1, except that the time of incubation varied.  $\circ$ , 18S rRNA from rat brain;  $\Delta$ , rat liver tRNA;  $\square$ , minus RNA.



other ribohomopolymer polymerases<sup>13</sup>. We believe that poly(U) polymerase has a primer specificity for larger RNA molecules, that it has some important role in the regulation of metabolism of larger RNAs, such as mRNA, rRNA or heterogeneous nuclear RNA (HnRNA).

Native DNA could act as a primer for the synthesis, but denatured DNA could not. The poly(U) polymerase may be capable of serving native DNA as a primer at a reduced rate, or a different enzymatic entity in the poly(U) polymerase fraction using native DNA as a primer, may be active. These points may be clarified by further purification of the enzyme.

The average length of the newly incorporated poly(U) sequence was determined. Table 3 shows the chain length of the product increasing during the 45 min incubation period. The products formed by the poly(U) polymerase clearly indicate that the reaction is sequential polymerisation.

Table 2 Primer specificity of poly(U) polymerase

| Primer                              | <sup>3</sup> H-UMP incorporated (pmol) | % Rate with <i>E. coli</i> rRNA |
|-------------------------------------|----------------------------------------|---------------------------------|
| 28S rRNA (rat brain)                | 32.2                                   | 77                              |
| 18S rRNA (rat brain)                | 35.9                                   | 85                              |
| Rat liver rRNA                      | 33.6                                   | 80                              |
| <i>E. coli</i> rRNA                 | 42.0                                   | 100                             |
| Q $\beta$ phage RNA                 | 33.0                                   | 79                              |
| Rat brain tRNA                      | 21.8                                   | 52                              |
| Rat liver tRNA                      | 13.4                                   | 32                              |
| <i>E. coli</i> tRNA                 | 19.1                                   | 45                              |
| Fragmented Q $\beta$ phage RNA (4S) | 14.7                                   | 35                              |
| Calf thymus DNA (native)            | 9.8                                    | 23                              |
| Calf thymus DNA (denatured)         | 0                                      | 0                               |
| Ribopolynucleotides                 |                                        |                                 |
| Poly(U)                             | 9.8                                    | 23                              |
| Poly(A)                             | 13.4                                   | 32                              |
| Poly(C)                             | 20.2                                   | 48                              |
| Poly(G)                             | 0                                      | 0                               |

Conditions of assay as described in Table 1, except that various nucleic acids were used as primers. Each primer was tested at a concentration of 5  $\mu$ g 0.25 ml<sup>-1</sup>. Q $\beta$  phage RNA was prepared as described previously<sup>13</sup>. RNA was isolated from brains (cerebrum) of 3-d-old Wistar rats. Brain was cut up with scissors and stirred for 30 min at 4 °C in a solution containing 0.1 M Tris-HCl, pH 7.4, 10 mM MgCl<sub>2</sub>, 0.5 mM 2-mercaptoethanol, 5  $\mu$ g ml<sup>-1</sup> DNase. This procedure was followed by adding 0.4% (w/v) sodium dodecyl sulphate (SDS) and incubating at 20 °C for 8 min. An equal volume of water-saturated phenol was added, and the mixture was shaken vigorously for 15 min at 4 °C. After centrifugation at 12,000g for 15 min, upper aqueous fraction was obtained. This phenol treatment was carried out four times, and extraction with ether was performed three times. The ether was flushed out with nitrogen gas, two volumes cold ethanol added; this was left to stand overnight at -20 °C. The mixture was centrifuged at 9,000g for 10 min, and the pellet was dissolved in TM (0.01 M Tris-HCl, pH 7.4, 5 mM MgCl<sub>2</sub>). After ethanol precipitation, the pellet was again dissolved in TM. Supernatant after centrifugation at 12,000g for 20 min was used for further purification. RNA fraction was incubated with 20  $\mu$ g ml<sup>-1</sup> DNase at 36 °C for 30 min and was purified by the SDS-phenol extraction method described above. To obtain rRNA and tRNA, extracted RNA fraction described above was layered on 25 ml 5–30% glycerol gradient (0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl, 5 mM EDTA) and ultracentrifuged in an SW 25.1 rotor at 21,000 r.p.m. for 18 h. Corresponding tRNA and rRNA fractions were collected by ethanol precipitation. RNAs from *E. coli* and rat liver were prepared as from brain. Fragmented Q $\beta$  phage RNA(4S) was prepared as follows. Intact Q $\beta$  phage RNA (1 mg ml<sup>-1</sup>) was hydrolysed in 0.1 M KOH for various incubation times at 36 °C, and the hydrolysate adjusted to pH 7.0 with 2.5% HClO<sub>4</sub>. Hydrolysate was then layered on 25 ml 5–30% glycerol gradient (0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl, 5 mM EDTA) and ultracentrifuged in an SW 25.1 rotor at 21,000 r.p.m. for 18 h. RNA sedimenting at the 4S position was collected by ethanol precipitation. To produce the new ends, the 4S RNA (0.36 mg ml<sup>-1</sup>) was incubated at 36 °C for 4 min with sufficient alkaline phosphatase from *E. coli* to release 0.45  $\mu$ mol p nitrophenol min<sup>-1</sup> as measured in the alkaline phosphatase assay described by Wilkie and Smellie<sup>9</sup>. Q $\beta$  phage RNA(4S) was prepared by SDS-phenol extraction method described above.

Table 3 Average chain length of polynucleotides synthesised *in vitro*

| Incubation time (min) | Ratio of radioactivity (Nucleoside / Nucleotide) | Average chain length |
|-----------------------|--------------------------------------------------|----------------------|
| 15                    | 7.65                                             | 9                    |
| 45                    | 19.12                                            | 20                   |

Ribohomopolymers were synthesised using 28S rRNA from rat brain (5  $\mu$ g per 0.25 ml) in 20-fold standard reaction mixtures at the time of incubation indicated and extracted with 0.1% SDS-phenol, precipitated with two volumes ethanol, dissolved in water and precipitated with 5% perchloric acid. Samples were then washed four times with 2.5% perchloric acid and finally with 66% ethanol-water. Alkaline hydrolysis was carried out with 0.33 N KOH at 36 °C for 18 h. Hydrolysed samples were chromatographed with standards of UMP and uridine on cellulose sheets (10×10 cm, Funakoshi) in a solution (isobutyric acid 13.2 ml, NH<sub>3</sub>OH 0.2 ml, H<sub>2</sub>O 6.6 ml). The appropriate areas were cut out and extracted with 0.01 N HCl. Radioactivity was then counted in a scintillation counter.

tion. Radioactivity of ribonucleoside 5'-phosphates (pppUp, ppUp or pUp) in the alkaline hydrolysate of radioactive products was not recovered, indicating that the poly(U) sequences were not synthesised *de novo* but formed by the successive addition of nucleotides to a pre-existing primer RNA.

To determine whether a sequential polymerisation occurs from the 3'-hydroxyl end of primer RNA or not, a nearest neighbour analysis using  $\alpha$ -<sup>32</sup>P-UTP was carried out. Q $\beta$  RNA and poly(C) were used as primer RNAs. Preparation of the products and thin-layer chromatography were carried out as described in the legend of Table 3. Using Q $\beta$  RNA, the ratio of radioactivity (UMP/(CMP+AMP)) increased during the 45 min incubation period from 5.92 (15 min) to 13.72 (45 min). At 45 min incubation, recovery of radioactivity in AMP, CMP and UMP was 4.6%, 2.2% and 93.3%, respectively. With poly(C), the ratio of radioactivity (UMP/CMP) also increased from 1.62 (15 min) to 3.20 (45 min). The 3'-hydroxyl end of Q $\beta$  RNA is known to be C-C-A (ref. 17). The recovery of radioactivity in CMP seems to be the result of removal of AMP during the preparation of Q $\beta$  RNA or incubation. This experiment clearly indicates that polymerisation occurs from 3'-hydroxyl end of RNA.

The precise biological implications of ribohomopolymer sequences in RNAs of eukaryotic cells remain obscure. It seems unlikely that the presence of poly(A) sequences is a prerequisite for translation<sup>15</sup>. It has recently been reported that mRNA having a poly(A) sequence on its 3'-end is more stable than poly(A)-free mRNA<sup>16</sup>. It is known that HnRNAs containing poly(U) sequences are unstable compared with those containing poly(A)<sup>1</sup>. The poly(U) polymerase catalysing sequential polymerisation from the 3'-hydroxyl end of RNA had a primer requirement for RNAs of larger size. We speculate that this enzyme has special significance in the regulation of metabolism of larger RNAs, such as mRNA, rRNA or HnRNA. Wilkie and Smellie detected a poly(U) polymerase in the microsomal fraction of rat liver<sup>8,9</sup>. The enzyme, however, catalyses the synthesis of short (two to five nucleotides) chains of poly(U) and has no primer specificity, unlike our poly(U) polymerase. We are in the process of looking for poly(U) polymerases having similar properties in organs other than the brain.

This work was supported in part by the Scientific Research Fund of the Ministry of Education of Japan, the Waksman Foundation of Japan and the Mitsubishi Foundation.

NOBUMICHI HOZUMI  
ICHIRO HARUNA  
ITARU WATANABE

Department of Molecular Biology,



KATSUHIKO MIKOSHIBA  
YASUZO TSUKADA

Department of Physiology,  
School of Medicine, Keio University,  
35 Shinanomachi, Shinjuku-ku, Tokyo, Japan

Received February 3; accepted June 2, 1975.

- <sup>1</sup> Lim, L., and Canellakis, E. S., *Nature*, **227**, 710–712 (1970).
- <sup>2</sup> Darnell, J. E., Wall, R., and Tushinski, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1321–1325 (1971).
- <sup>3</sup> Edmonds, M., Vaughan, M. H., and Nakazato, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1336–1340 (1971).
- <sup>4</sup> Burdon, R. H., and Shenkin, S., *FEBS Lett.*, **24**, 11–14 (1972).
- <sup>5</sup> Lim, L., Canellakis, Z. N., and Canellakis, E. S., *Biochim. biophys. Acta*, **209**, 112–127 (1970).
- <sup>6</sup> Niessing, J., and Sekeris, C. E., *FEBS Lett.*, **22**, 83–88 (1972).
- <sup>7</sup> Winters, M. A., and Edmonds, M., *J. biol. Chem.*, **248**, 4756–4762 (1973).
- <sup>8</sup> Wilkie, N. M., and Smellie, R. M. S., *Biochem. J.*, **109**, 485–494 (1968).
- <sup>9</sup> Wilkie, N. M., and Smellie, R. M. S., *Biochem. J.*, **109**, 229–238 (1968).
- <sup>10</sup> Niessing, J., and Sekeris, C. E., *Nature new Biol.*, **243**, 9–12 (1973).
- <sup>11</sup> Mikoshiba, K., Tsukada, Y., Haruna, I., and Watanabe, I., *Nature*, **249**, 445–448 (1974).
- <sup>12</sup> Haruna, I., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 579–587 (1965).
- <sup>13</sup> Burkard, G., and Keller, E. B., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 389–393 (1974).
- <sup>14</sup> Adesnik, M., Salditt, M., Thomas, W., and Darnell, J. E., *J. molec. Biol.*, **71**, 21–30 (1972).
- <sup>15</sup> Williamson, R., Crossley, J., and Humphries, S., *Biochemistry*, **13**, 703–707 (1974).
- <sup>16</sup> Huez, G., et al., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3143–3146 (1974).
- <sup>17</sup> Goodman, H. M., Billetter, M. A., Hindley, J., and Weissmann, C., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 921–928 (1970).

## N-terminal sequences of secretory piece and of $\alpha$ chains of different allotype in rabbit secretory IgA

THE translocation hypothesis<sup>1,2</sup> states that in the process of antibody synthesis any one of a number of immunoglobulin heavy chain *V* genes is available for union with any of the class-specific *C* genes. Whereas almost all structural data support the widely held view that all *V* genes are able to translocate to any of the class-specific *C* genes, some data suggest that this may not be the case. Wilkinson<sup>3</sup> found the N-terminal sequence PCA-Ser-Ser (PCA, pyrrolidone carboxylic acid) to be present in a rabbit  $\alpha$  chain preparation but absent in pooled rabbit  $\gamma$  chains. Other workers<sup>4,5</sup> have described an N-terminal heavy chain sequence apparently confined to the IgM class of human immunoglobulins.

We have reinvestigated the former result in view of the finding that secretory piece (SP), a polypeptide chain present in secretory IgA (sIgA) but absent from IgG, has a molecular weight of 60,000–70,000 (refs 6 and 7), in both the native and fully reduced state, rather than being a disulphide-linked dimer with subunits of molecular weight 25,000 (ref. 8). Wilkinson isolated his  $\alpha$  chain preparation after complete reduction of rabbit sIgA using gel filtration with Sephadex G-200; secretory piece could have contaminated this preparation and provided the novel N-terminal peptide. The fact that the yield of the PCA-Ser-Ser peptide from the  $\alpha$  chain preparation was 14–16% (ref. 3) and that the ratio of  $\alpha$  chain to SP in the intact molecule is 4:1 (ref. 7) suggested that the above reasoning may be correct.

Wilkinson<sup>3</sup> compared the N-terminal PCA peptides of rabbit  $\alpha$  chains of allotype a1 and a3 with peptides isolated in an earlier study of  $\gamma$  chains of the two allotypes<sup>9</sup>. The *a* locus allotypic specificities are located in the *V* region of rabbit heavy chains of all the known classes and have been shown for  $\gamma$  chains to be associated with multiple differences in primary structure<sup>9,10</sup>. Differences seen in the N-terminal PCA peptides of rabbit  $\gamma$  chain<sup>9</sup> (see Table 1) of the two allotypes proved similar in  $\alpha$  chains of different allotype except for the additional peptide PCA-Ser-Ser found in  $\alpha$  chain of both a1 and a3 specificity in a yield of 0.15 mol mol<sup>-1</sup>.

In this study, preliminary experiments on totally reduced

and alkylated sIgA showed, by alkaline urea and SDS gel electrophoresis, that the  $\alpha$  chain fraction was indeed contaminated by secretory piece. We therefore tried to separate the  $\alpha$  chain fraction from secretory piece. sIgA was isolated from clarified rabbit colostrum using the method of Cebra and Robbins<sup>11</sup> with slight modification. Attempts to separate  $\alpha$  chain from SP after complete reduction and alkylation of sIgA, either by gel filtration, preparative gel electrophoresis or immunoadsorbents, proved fruitless (A.P.J. and L.E.M., unpublished). We purified secretory piece by taking advantage of the fact that not all SP is covalently bound to the sIgA molecule<sup>8</sup>. Thus, in our preparation, gel filtration in 3 M guanidine<sup>8</sup> released about 50% of the SP from sIgA; this fraction was pure on SDS gel electrophoresis and was free of any light chain contamination as judged by anti-light chain antisera. The N-terminal half of the  $\alpha$  chain was eventually freed of the remaining covalently bound SP by peptic digestion of sIgA for 20 h at 37 °C in 0.1 M glycine-HCl pH 2.4 (A.P.J. and L.E.M., unpublished) followed by gel filtration on G-200 in 0.1 M phosphate buffer, pH 6.8. Such treatment digested SP and the  $\alpha$  chain Fc region into peptides releasing a large fragment, F(ab')<sub>2a</sub>, equivalent in component structure to the well defined F(ab')<sub>2</sub> fragment released by peptic digestion of IgG (ref. 12). The F(ab')<sub>2a</sub> fraction was unreactive with antisera to SP.

The isolated SP and F(ab')<sub>2a</sub> from both allotypes a1 and a3 were completely reduced and alkylated, digested with Pronase and the PCA peptides collected in the unretarded fraction from Dowex 50 ( $\times 2$ ; H<sup>+</sup> form). Purification of the

Table 1 PCA peptides of Aa1, Aa3 F(ab')<sub>2a</sub> and SP\*

|                 | $\gamma$ chain <sup>9</sup> |      | $\alpha$ chain |      | SP   |
|-----------------|-----------------------------|------|----------------|------|------|
|                 | Aa1                         | Aa3  | Aa1            | Aa3  |      |
| PCA-Ser-Val-Glu | 0.56                        | —    | 0.61           | —    | —    |
| PCA-Ser-Leu-Glu | 0.14                        | 0.50 | 0.15           | 0.43 | —    |
| PCA-Glu-Gln     | —                           | 0.26 | —              | 0.27 | —    |
| PCA-Ser-Ser     | —                           | —    | —              | —    | 0.65 |
| Total           | 0.70                        | 0.76 | 0.76           | 0.70 | 0.65 |

\*Yields as mol peptide per mol polypeptide chain.

PCA peptides from the Dowex 50 fractions was carried out using gel filtration and paper electrophoresis as described by Wilkinson<sup>9</sup>.

The N-terminal PCA peptides isolated from SP, Aa1 F(ab')<sub>2a</sub> and Aa3 F(ab')<sub>2a</sub>, are described in Table 1 and compared with the  $\gamma$  chain peptides described previously<sup>9</sup>. Peptide yields were calculated using the same assumptions used in the earlier studies<sup>9,10</sup>. The yields of the peptides PCA-Ser-Val-Glu and PCA-Ser-Leu-Glu (Table 1) from the F(ab')<sub>2a</sub> fragment of a1 allotype proved similar to those derived from  $\gamma$  chain as did the yields of peptides PCA-Ser-Leu-Glu and PCA-Glu-Gln from Aa3 F(ab')<sub>2a</sub> (ref. 9). There was no indication of the peptide PCA-Ser-Ser in either F(ab')<sub>2a</sub> digest; but this peptide was isolated in good yield (65%) from the SP digest (Table 1). Although, unlike Wilkinson<sup>3</sup>, we digested light chains together with the N-terminal region of  $\alpha$  chain, we isolated no PCA peptides other than those normally found in  $\gamma$  chain. This reflects the fact that the vast majority of the rabbit light chain fraction is of the unblocked *K* type.

We conclude that the N-terminal sequence of rabbit secretory piece is PCA-Ser-Ser and that the earlier report<sup>3</sup> that this represented a sequence unique to rabbit  $\alpha$  chain *V* region was incorrectly made because of the inability to separate these two polypeptide chains before characterization. Although our finding does not rule out the possibility that some *V* region subgroups may be confined to a certain

class of heavy chain it does remove some structural evidence against a simple translocation hypothesis.

We thank Professor R. R. Porter for discussion and Mr A. C. Willis for technical assistance.

ALAN P. JOHNSTONE  
LAWRENCE E. MOLE

MRC Immunochemistry Unit,  
Department of Biochemistry,  
University of Oxford, Oxford OX1 3QU, UK

Received May 5; accepted May 21, 1975.

- <sup>1</sup> Dreyer, W. J., and Bennett, J. C., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 864-869 (1965).
- <sup>2</sup> Gally, J. A., and Edelman, G. M., *Nature*, **227**, 341-348 (1970).
- <sup>3</sup> Wilkinson, J. M., *Nature*, **223**, 616-617 (1969).
- <sup>4</sup> Bennett, J. C., *Biochemistry*, **7**, 3340-3344 (1968).
- <sup>5</sup> Köhler, H., Shimizu, A., Paul, C., Moore, V., and Putnam, F. W., *Nature*, **227**, 1318-1320 (1970).
- <sup>6</sup> Halpern, M. S., and Koshland, M. E., *Nature*, **228**, 1276-1278 (1970).
- <sup>7</sup> O'Daly, J. A., and Cebra, J. J., *Biochemistry*, **10**, 3843-50 (1971).
- <sup>8</sup> Cebra, J. J., and Small, P. A., *Biochemistry*, **6**, 503-512 (1967).
- <sup>9</sup> Wilkinson, J. M., *Biochem. J.*, **112**, 173-185 (1969).
- <sup>10</sup> Mole, L. E., Jackson, S. A., Porter, R. R., and Wilkinson, J. M., *Biochem. J.*, **124**, 301-318 (1971).
- <sup>11</sup> Cebra, J. J., and Robbins, J. B., *J. Immun.*, **97**, 12-24 (1966).
- <sup>12</sup> Nisonoff, A., and Dixon, D. J., *Biochemistry*, **3**, 1338-1342 (1964).

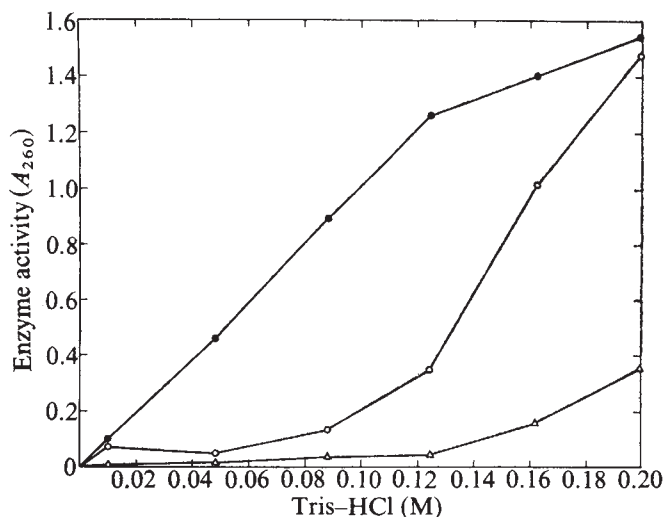
## Possible role for poly(A) as an inhibitor of endonuclease activity in eukaryotic cells

A NUMBER of laboratories have reported the isolation from eukaryotic cells of mRNAs linked covalently at their 3'-OH termini to polyadenylic acid (poly(A))<sup>1-4</sup>. The chain of adenylic acid residues has been found also at the 3' termini of RNAs isolated from several well characterised viruses<sup>5-7</sup>. Although considerable evidence exists to suggest that the insertion of poly(A) on the RNA chains destined to become mRNA is a post-transcriptional event<sup>8</sup>, there is at present little understanding of the part poly(A) plays in the subsequent stages of protein synthesis.

In this communication we should like to suggest a possible role for the polynucleotide in maintaining the structural and functional integrity of mRNA. The role is based on the observation that poly(A) can act as a general inhibitor of endonuclease activity and in so doing can prevent the degradation of mRNA in the cytoplasm. The enzymes used in this study have been isolated from microbial and from human sources. Their purification and properties have been described elsewhere<sup>9-11</sup>.

Poly(A), much like the ordered polynucleotide, polyguanylic acid (poly(G)), was found to act as a strong inhibitor of a microbial ribonuclease isolated from *Citrobacter* sp.<sup>9,12</sup>. The inhibition of enzyme activity induced by poly(G) can be only partially reversed by increasing concentrations of buffer. In contrast, the inhibition induced by poly(A) is strongly dependent on the ionic strength of the buffering mediums (Fig. 1). As the molar concentration of buffer is increased, the inhibitory effect of poly(A) is reduced until, at the highest buffer concentration studied, the presence of the polynucleotide has relatively little effect on enzyme activity (Fig. 1). On the other hand, poly(G), in the highest molarity buffer, remains a powerful enzyme inhibitor.

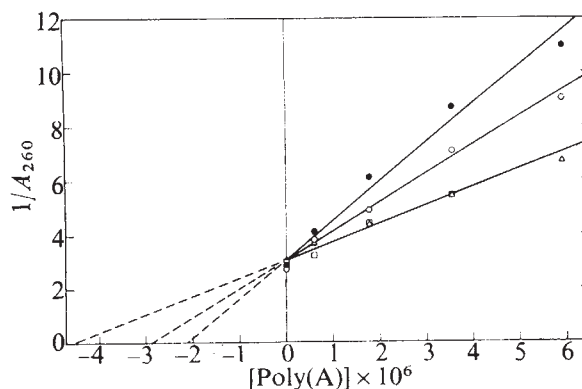
Since there is considerable variation in the number of nucleotide residues in poly(A) at the end of individual mRNAs<sup>14</sup>, it was of interest to study the relationship between chain length and the inhibitory properties of the polynucleotide. As shown in Fig. 2, the two shortest chains with an average monomer length of 25 (25) and 45 (45) residues respectively inhibited enzyme activity to essentially the same degree ( $I_{50}=4.5 \times 10^{-6}$  M). The intermediate-sized polymer with an average monomer length of 90 residues



**Fig. 1** Reversal by increasing concentrations of Tris-HCl buffer of polynucleotide-induced inhibition of *Citrobacter* RNase activity. Enzyme activity was measured in 1 ml of a reaction mixture consisting of varying concentrations of Tris-HCl buffer (pH 7.1), 0.5  $\mu$ mol of poly(U) and 15 U of enzyme. After incubation for 5 min at 37°C, the reaction was stopped by the addition of 1 ml of 2 N perchloric acid containing 20 mM lanthanum nitrate and the reaction vessel was placed in an ice bath for 20 min. The mixture was then clarified by centrifugation and the  $A_{260}$  of the acid-soluble nucleotides measured<sup>13</sup>. When the effects of the polynucleotide on enzyme activity were to be studied, poly(A) ( $5 \times 10^{-6}$  M) and poly(G) ( $2.5 \times 10^{-6}$  M) were added, before the addition of enzyme, to separate reaction mixtures. RNase activity measured in a control vessel (●) and in the presence of poly(A) (○) and poly(G) (△).

(90) was a better inhibitor ( $I_{50}=2.9 \times 10^{-6}$  M) than either of the smaller chains. Finally, the polymer having the highest molecular weight (average monomer length of 315) proved to be the best inhibitor of all ( $I_{50}=2.1 \times 10^{-6}$  M). Although greater inhibition of enzyme activity was observed as the average chain length of poly(A) was lengthened, the increase was gradual and did not seem to be so strongly controlled by chain length that inhibition was a step function of average molecular weight in the region available for study. The gradual decrease in inhibitory properties associated with decreasing chain length suggested that below a certain size, inhibition by poly(A) would not occur. The

**Fig. 2** Inhibition of *Citrobacter* RNase activity by various molecular weight fractions of poly(A). Average monomer length (Miles Laboratories) based on intrinsic viscosity and sedimentation velocity, 50% range: □, 25 (25-51); △, 45 (39-82); ○, 90 (62-108); ●, >300. Enzyme activity was measured as in Fig. 1, except that 55  $\mu$ mol of Tris-HCl buffer (pH 7) was used in all the determinations.





**Table 1** Reversal by polyamines of poly(A)-induced inhibition of RNase activity

| RNase              | Concentration of poly(A) | —    | MgCl <sub>2</sub> | Enzyme activity ( $\Delta A_{260}$ ) of cation added |            |          |
|--------------------|--------------------------|------|-------------------|------------------------------------------------------|------------|----------|
|                    |                          |      |                   | Putrescine                                           | Spermidine | Spermine |
| <i>Citrobacter</i> | —                        | 1.31 | 1.35              | 1.29                                                 | 1.52       | 1.49     |
|                    | $5.0 \times 10^{-6}$ M   | 0.29 | 0.30              | 0.33                                                 | 1.25       | 1.50     |
| Human plasma       | —                        | 2.10 | 2.27              | 2.39                                                 | 2.47       | 2.26     |
|                    | $1.5 \times 10^{-6}$ M   | 0.78 | 0.78              | 0.97                                                 | 2.23       | 2.10     |
| Human spleen       | —                        | 1.05 | 1.11              | 1.38                                                 | 1.43       | 1.21     |
|                    | $2.0 \times 10^{-6}$ M   | 0.08 | 0.78              | 0.50                                                 | 1.44       | 1.27     |

*Citrobacter* RNase activity was measured as in Fig. 1, except that 40  $\mu$ mol of potassium phosphate buffer (pH 7.1), were used in place of Tris-HCl. When the effects on enzyme activity of poly(A) and/or cations listed above were studied, 0.005  $\mu$ mol of poly(A), followed by 0.5  $\mu$ mol of cation were added before the addition of enzyme to reaction mixtures similar to those described above. The hydrolysis of poly(C) (1.5  $\mu$ mol) used as a measure of the activity of human plasma RNase was assayed as described elsewhere<sup>10</sup>. Here also poly(A) (0.015  $\mu$ mol) and 0.5  $\mu$ mol of cation were added to the reaction mixtures before the addition of 21 U of enzyme. The human spleen RNase activity<sup>11</sup> was measured in a reaction mixture (1 ml) containing 1  $\mu$ mol of potassium phosphate buffer (pH 6.2), 40  $\mu$ mol of poly(U) and 10 U of human spleen RNase. After incubation for 15 min at 37 °C, enzyme activity was measured by the production of acid-soluble nucleotides<sup>14</sup>. Poly(A) (0.002  $\mu$ mol) and cations (0.4  $\mu$ mol) were added also to reaction mixtures before the addition of enzyme.

critical chain size could not be determined, however, because of the lack of availability of a graduated series of smaller polymer chains.

Another characteristic of polynucleotide inhibition of RNase activity is that it is readily reversible when the highly charged polyamines are added. As is apparent from Table 1, the poly(A)-induced inhibition of a number of RNases can be reversed with these substances. At concentrations of poly(A) sufficient to cause severe reductions in enzyme activity of both the *Citrobacter* and a human plasma RNase, only spermine or spermidine could restore activity to uninhibited levels. Putrescine and a variety of metal cations tested (represented by Mg<sup>2+</sup> in Table 1) were ineffective in reversing the inhibition. Enzyme activity of a third RNase (from human spleen), although completely inhibited by poly(A), could be restored by spermine or

spermidine, and to a lesser extent by Mg<sup>2+</sup> and putrescine.

The influence of poly(A) on RNase activity was of interest from the point of view of enzyme stability as well. The association between polynucleotide and enzyme was found to protect the RNase from thermal inactivation. Thus, in the absence of the polynucleotide, the half life of a human liver RNase (unpublished results) was 20 min (Fig. 3), whereas in the presence of poly(A), the half life of the enzyme was found to be increased by as much as fivefold.

The results of these studies suggest a model whereby poly(A), at the terminus of a mRNA, acts to prevent the degradation of the mRNA. The polynucleotide would do this by binding to, and thus inhibiting, any RNase in the vicinity of the mRNA. With this in mind, it is entirely conceivable that translation can occur with an RNase bound to the poly(A) moiety at the end of the mRNA chain. This would also help explain the long lived mRNAs of eukaryotic cells in contrast to the rapidly degraded ones of prokaryotes<sup>15</sup>. Degradation of the mRNA can, nonetheless, be assured by any of a number of means that would dissociate the complex formed between the mRNA·poly(A) and the RNase. An increase in ionic strength or in polyamine concentration would, of course, be examples of factors causing dissociation of the complex and thereby reversing the inhibition of the enzymes. The association between RNase and polynucleotide also provides a means of storing the enzyme for long periods, thus ensuring that the protein, although quiescent, is not denatured and is immediately active when needed. In effect, the mRNA·poly(A) chain might carry its own "self-destruct" mechanism. Studies are in progress attempting to verify the model *in vivo*.

CARL C. LEVY

MORTON SCHMUKLER

JOSEPH J. FRANK

TIMOTHY P. KARPETSKY

PHILLIP B. JEWETT

PHILIP A. HIETER

STEPHEN M. LE GENDRE

ROBERT G. DORR

Enzymology and Drug Metabolism Section,  
Laboratory of Pharmacology,  
Baltimore Cancer Research Center,  
National Cancer Institute,  
National Institute of Health,  
Baltimore, Maryland 21211

Received April 10; accepted June 2, 1975.

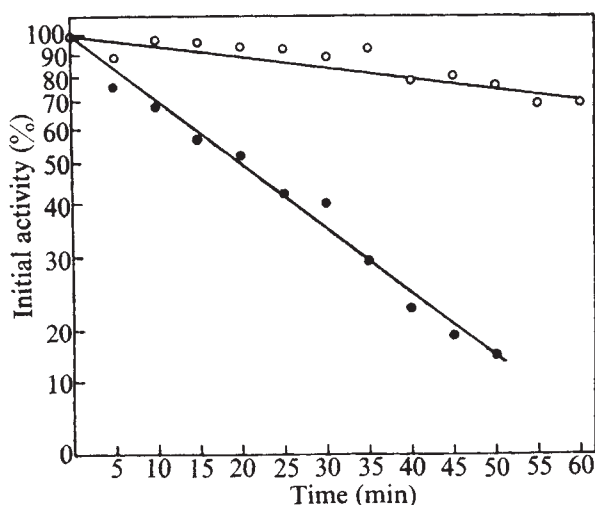
<sup>1</sup> Kates, J., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 743-762 (1970).

<sup>2</sup> Mendecki, J., Lee, S.-Y., and Brawerman, G., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1331-1335 (1971).

<sup>3</sup> Edmonds, M., Vaughan, M. H., and Nakazato, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1336-1340 (1971).

<sup>4</sup> Darnell, J. E., Wall, R., and Tushinski, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1321-1325 (1971).

**Fig. 3** The effect of poly(A) on the thermal inactivation of human liver RNase. To one of two flasks, each containing 40 U of human liver RNase in 0.1 M potassium phosphate buffer (pH 6.4), 0.25  $\mu$ mol of poly(A) were added. The final volume in each vessel was brought to 1 ml and the mixtures incubated at 37 °C. Aliquots (25  $\mu$ l) withdrawn from each vessel at 5-min intervals were tested for enzyme activity in a reaction mixture containing 0.25 mg of yeast RNA and 100  $\mu$ mol of potassium phosphate buffer (pH 6.4)<sup>11</sup>. After 15 min at 37 °C, the reaction was stopped by the addition of 1 ml of 2 N perchloric acid and the mixture chilled for 20 min. The  $A_{260}$  of acid-soluble nucleotides was measured after clarification of the mixture by centrifugation. Enzyme activity (●); enzyme activity in presence of poly(A) (○).



- <sup>5</sup> Perlman, S., Abelson, H. T., and Penman, S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 350-353 (1973).  
<sup>6</sup> El Manna, M. M., and Bruening, G., *Virology*, **56**, 198-206 (1973).  
<sup>7</sup> Spector, D. H., and Baltimore, D., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2983-2987 (1974).  
<sup>8</sup> Adesnik, M., Salditt, M., Thomas, W., and Darnell, J. E., *J. molec. Biol.*, **71**, 21-30 (1972).  
<sup>9</sup> Levy, C. C., Mitch, W. E., and Schmukler, M., *J. biol. Chem.*, **248**, 5712-5719 (1973).  
<sup>10</sup> Schmukler, M., Jewett, P. B., and Levy, C. C., *J. biol. Chem.*, **250**, 2206-2212 (1975).  
<sup>11</sup> Delaney, R., *Biochemistry*, **2**, 438-444 (1963).  
<sup>12</sup> Levy, C. C., Hieter, P. A., and LeGendre, S. M., *J. biol. Chem.*, **249**, 6782-6789 (1974).  
<sup>13</sup> Kalnitsky, G., Hummel, J. P., and Dierks, C. J., *J. biol. Chem.*, **234**, 1512-1516 (1959).  
<sup>14</sup> Brawerman, G., *A. Rev. Biochem.*, **43**, 621-642 (1974).  
<sup>15</sup> Morse, D. E., Mosteller, R., Baker, R. F., and Yanofsky, C., *Nature*, **223**, 40-43 (1969).

## Stereoselective blockade of the dopamine receptor and the X-ray structures of $\alpha$ and $\beta$ -flupenthixol

THE introduction of neuroleptics of the phenothiazine and thioxanthene classes has revolutionised the treatment of schizophrenia and there is increasing evidence<sup>1-3</sup> that links this antipsychotic action to a blockade of dopamine receptors in the brain. The thioxanthene compounds are of particular interest as, because of the presence of an exocyclic double bond and a substituent in the tricyclic nucleus, they exist as *cis-trans* geometric isomers about the double bond with respect to the ring substituent (Fig. 1). Virtually all the neuroleptic activity is confined to the *cis* isomer<sup>4</sup>.

One of the most potent drugs in this group is flupenthixol (Fig. 1); a mixture of the *cis* ( $\alpha$ ) and *trans* ( $\beta$ ) isomers is a clinically efficacious antipsychotic agent<sup>5</sup> but in dopamine receptor related animal tests the  $\alpha$  isomer is much more potent than the  $\beta$  form<sup>4</sup> (Table 1). In *in vitro* studies using the dopamine sensitive adenyl cyclase system of the rat corpus striatum as a model of the dopamine receptor, the  $\alpha$  isomer is a very potent blocker of the dopamine induced enzyme stimulation whereas the  $\beta$  isomer is almost inactive<sup>6</sup> (Table 1).

In an attempt to explain how chlorpromazine is able to block dopamine receptors, attention has been focused on a possible spatial complementary relationship between certain

portions of the crystal structure of chlorpromazine and dopamine<sup>7</sup>. Further support for this concept has been obtained from a detailed conformational analysis of 15 drugs of the tricyclic class<sup>8</sup>. As flupenthixol displays such a marked degree of stereoselectivity in blocking dopamine receptors, it is believed that X-ray analysis of the two isomers could provide additional insight into the various interatomic distance and overall conformational requirements for effective dopamine receptor antagonism. Although the *cis* and *trans* geometrical isomers will have differing physical properties this is unlikely to be the sole factor accounting for their differential pharmacological potency, as neuroleptics possessing optically active centres, such as butaclamol, also show a high degree of stereoselectivity<sup>7-9</sup>.

Crystals of each isomer were obtained and the cell dimensions and other related parameters for each compound were found:

$\alpha$ -flupenthixol: orthorhombic; *Pbca*;  $a = 8.827$ ,  $b = 20.048$ ,  $c = 20.251$  Å; 8 molecules per cell; final *R* factor 0.066 for 1,094 reflections.

$\beta$ -flupenthixol: monoclinic; *P2<sub>1</sub>/c*;  $a = 9.011$ ,  $b = 15.218$ ,  $c = 18.493$  Å;  $\beta = 120.37^\circ$ ; 4 molecules per cell; current *R* factor 0.072 for 2,308 reflections.

Diffraction data for both compounds were collected using Cu-K $\alpha$  radiation to a resolution of 0.89 Å ( $2\theta_{\max}$  of  $120^\circ$ ) and each structure was solved by the application of locally programmed (G. M. Sheldrick, unpublished) multiresolution direct method techniques. Complete details of these structures will be published elsewhere. Figure 1 shows a general view of each isomer based on approximately equivalent orientations of the tricyclic nucleus. The *cis* or *trans* configuration at the exocyclic double bond is clearly apparent and each side chain exhibits markedly different conformation although the piperazine rings favour the -CF<sub>3</sub> 'side' of the molecule in either case. Principal torsion angles for the side chain are shown in Fig. 1. The dihedral angles between the aromatic ring mean planes are  $152^\circ$  and  $143^\circ$  for  $\alpha$  and  $\beta$ -flupenthixol respectively.

Various lines of evidence support the concept that the preferred conformation of dopamine at its receptor site is the fully extended *trans* form<sup>7</sup>; therefore, it has been suggested that the binding site for dopamine's amino group is about 5.1 Å from the centre of the aromatic ring<sup>7</sup>. There is also evidence that the dopamine receptor antagonism by the neuroleptics is

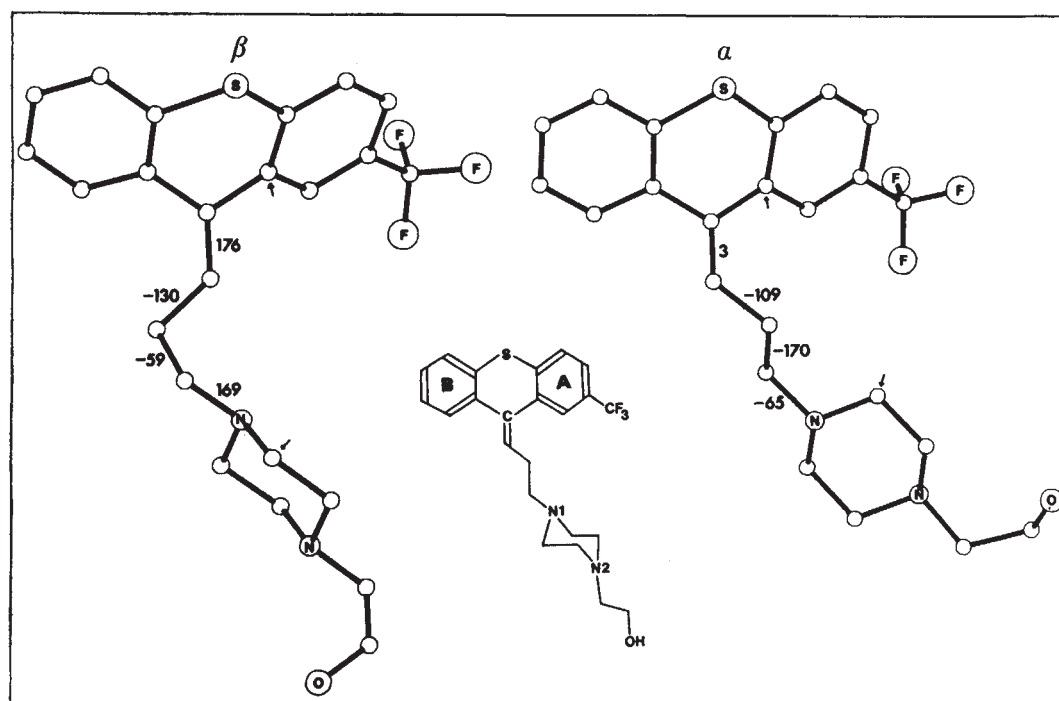


Fig. 1  $\alpha$  and  $\beta$  isomers of flupenthixol computed from the crystallographic coordinates, together with a chemical representation of the molecules (shown in the  $\alpha$  form) on which is indicated labelling referred to in the text. The values of some principal torsion angles (in degrees) for each isomer are shown alongside the bond to which they refer, and where an ambiguity in definition occurs, an arrow marks atoms used in the calculations.



**Table 1** Comparison of the effects of  $\alpha$  and  $\beta$ -flupenthixol on various dopamine receptor systems

|                        | Antagonism of apomorphine induced stereotypy in rats ED <sub>50</sub> (mg kg <sup>-1</sup> ) (ref. 4) | Antagonism of amphetamine induced stereotypy in rats ED <sub>50</sub> (mg kg <sup>-1</sup> ) (ref. 4) | K <sub>i</sub> for dopamine sensitive adeny cyclase antagonism <sup>6</sup> |
|------------------------|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| $\alpha$ -flupenthixol | 0.3                                                                                                   | 0.07                                                                                                  | $1.0 \times 10^{-9}$ (M)                                                    |
| $\beta$ -flupenthixol  | > 80                                                                                                  | > 160                                                                                                 | $> 5 \times 10^{-6}$ (M)                                                    |

competitive<sup>10,11</sup> and for simplicity this can be considered to indicate occupation, by the neuroleptic, of the same site as the natural agonist. Thus it is of interest to contrast significant intramolecular distances between  $\alpha$  and  $\beta$ -flupenthixol. In  $\alpha$ -flupenthixol the distances of N1 from the centres of the two aromatic rings A and B (Fig. 1) are 5.82 Å and 7.46 Å respectively, and the corresponding distances from N2 are much longer, with N2-A = 7.75 Å and N2-B = 10.26 Å.

This suggests that N1 could bind at the receptor site normally occupied by the amino group of dopamine whereas the aromatic ring A would interact at the site normally influenced by the benzene ring of dopamine. The importance of N1 rather than N2 as the atom involved in the antagonistic binding is supported by comparison with similar distances found in  $\alpha$ -chlorprothixene<sup>7,12</sup>, a pharmacologically active compound which, however, has only a single N atom in the side chain. There is a marked difference in the almost inactive  $\beta$ -flupenthixol where N1-A = 6.09 Å, N1-B = 6.46 Å, N2-A = 8.24 Å and N2-B = 9.30 Å, indicating that the  $\alpha$ -isomer exhibits a better 'fit' as a dopamine antagonist. Extrapolation from results of crystal structure determinations to the conformation of molecules in physiological conditions has always to be interpreted with caution, but the absence of any unusually close intermolecular contacts in the crystal structures suggests that the observed conformation is not the result of packing forces and that a closely similar conformation could be found *in vivo*.

The A and B rings of the tricyclic nucleus are differentiated by the presence of the -CF<sub>3</sub> moiety. This group is of evident importance for pharmacological activity although its mode of action is not clearly understood. It is perhaps of interest to note that the molecular asymmetry introduced in the tricyclic nucleus by the -CF<sub>3</sub> group is enhanced in the  $\alpha$  form where the difference in the distance of N1 from the centres of the aromatic rings is greater than in the  $\beta$  form<sup>7</sup>.

The X-ray studies reported here were carried out on the free bases. An X-ray analysis of the dihydrochloride salts is in progress to investigate the effect of positive charges on the nitrogen atoms of the piperazine ring on the conformation of these molecules.

We thank the MRC for financial support and the SRC for provision of the diffractometer. Olga Kennard is on the External Staff of the MRC. Dr I. Møller-Nielsen of H. Lundbeck and Co., Denmark, provided the specimen crystals.

MICHAEL L. POST  
OLGA KENNARD

University Chemical Laboratory,  
Lensfield Road, Cambridge CB2 1EW, UK

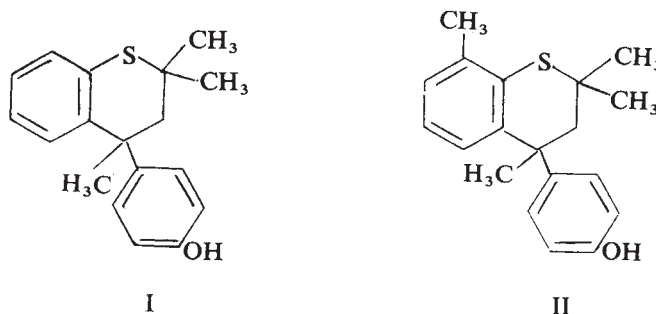
ALAN S. HORN

Neurochemical Pharmacology Unit,  
Department of Pharmacology,  
Medical School, Hills Road, Cambridge, UK

- <sup>6</sup> Miller, R. J., Horn, A. S., and Iversen, L. L., *Mol. Pharmacol.*, **10**, 759-766 (1974)  
<sup>7</sup> Horn, A. S., Post, M. L., and Kennard, O., *J. Pharm. Pharmacol.* (in the press).  
<sup>8</sup> Miller, R. J., Horn, A. S., and Iversen, L. L., *J. Pharm. Pharmacol.*, **27**, 212-213 (1975).  
<sup>9</sup> Bruderlein, F. T., Humber, L. G., and Voith, K., *J. med. Chem.*, **18**, 185-188 (1975).  
<sup>10</sup> York, D. H., *Brain Res.*, **37**, 91-99 (1972).  
<sup>11</sup> Clement-Cormier, Y. C., Kebejian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1113-1117 (1974).  
<sup>12</sup> Post, M. L., Kennard, O., and Horn, A. S., *Acta crystallogr.* **B30**, 1644-1646 (1974).

## Alteration of cavity geometry by structural modification of clathrate host molecules

IN the course of systematic studies<sup>1</sup> of the synthesis and properties of clathrate inclusion compounds we found that compound II, formed by adding a methyl group to the clathrating compound I, also exhibits clathrating properties. It was evident, however, both from preliminary crystallographic measurements and from their very different selectivities towards guest molecules that the cavities in the host structure of I and II differ markedly<sup>2</sup>. We present here details of the cavities in II and compare them with those reported earlier for I (ref. 3).



The cyclooctane clathrate of II, which was selected for crystallographic study, is trigonal with a unit cell ( $a = 33.629(9)$ ;  $c = 8.239(3)$  Å) containing 18 molecules of C<sub>10</sub>H<sub>22</sub>OS. A host-guest (cyclooctane) ratio of 4.5:1 was measured by <sup>1</sup>H nuclear magnetic resonance for a CDCl<sub>3</sub> solution. The space group is either R3 or R<sub>3</sub><sup>2</sup>; (ref. 4) the centrosymmetric space group (R3) was chosen by analogy to the structure of the clathrates of I (ref. 3) and this choice has been justified by successful analysis. The structure was solved by direct methods using 1,915 independent reflections measured with Mo-K $\alpha$  radiation on a Hilger-Watts automatic diffractometer, and refined to a current  $R$  value of 0.119. All 22 of the hydrogen atoms of the host molecule have been located and allowed for; it has not yet been possible, however, to allow effectively for the guest molecules and this should allow further reduction in  $R$ .

Figure 1 illustrates the general similarity of the clathrate host structures of I and II. Both structures contain groups of six molecules the hydroxyl groups of which are linked by

Received April 14; accepted May 15, 1975.

<sup>1</sup> Horn, A. S., and Snyder, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2325-2328 (1971).

<sup>2</sup> Matthyssse, S., *Fedn Proc.*, **32**, 200-205 (1973).

<sup>3</sup> Snyder, S. H., Banerjee, S. P., Yamamura, H. I., and Greenburg, D., *Science*, **184**, 1243-1253 (1974).

<sup>4</sup> Møller-Nielsen, I., et al., *Acta pharmac. tox.*, **33**, 353-362 (1973).

<sup>5</sup> Gottfries, C. G., *Br. J. Psychiat.*, **119**, 547-548 (1971).

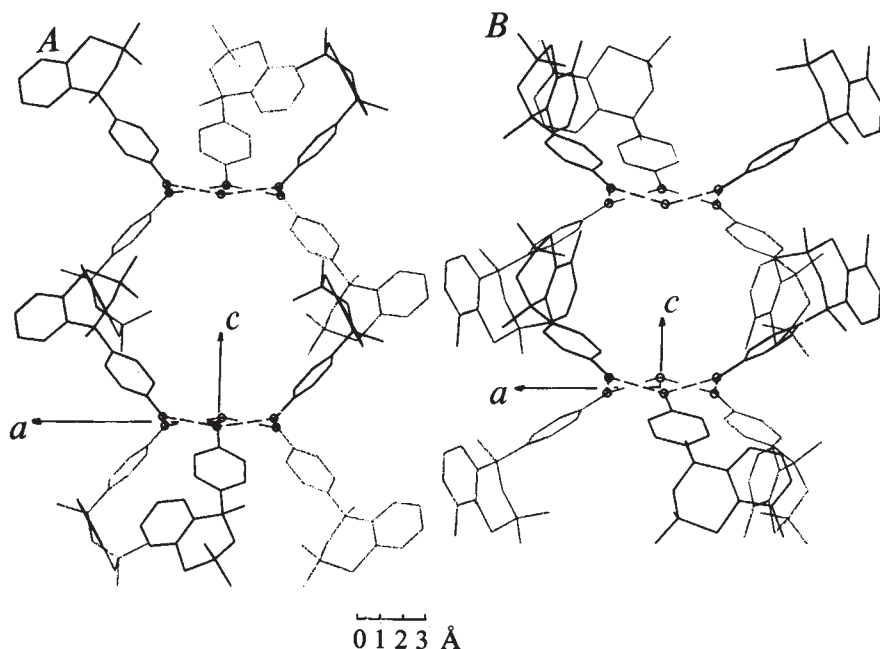
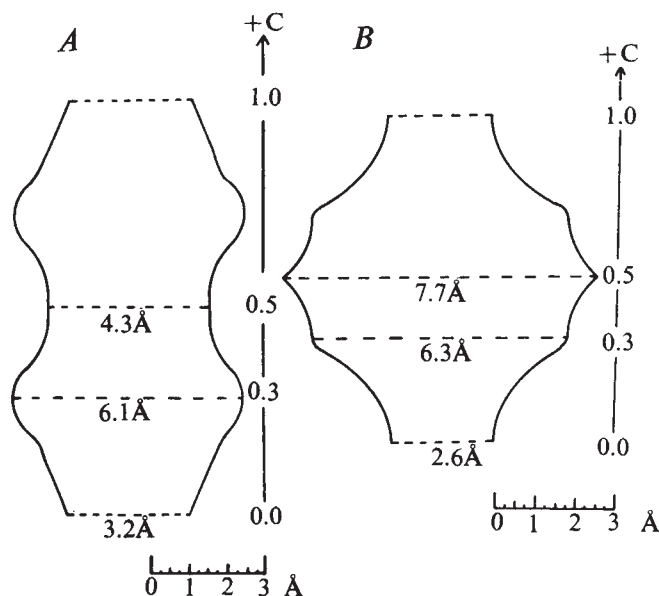


Fig. 1 *A*, Structure of compound I looking on to the *a*-*c* plane. The guest molecule, 2,5,5-trimethylhex-3-yn-2-ol, is not shown. *B*, Structure of compound II looking on to the *a*-*c* plane. The (disordered) cyclooctane guest is not shown. In both cases, two host molecules which lie above and below the cavity as viewed in the direction, have been excluded, apart from their hydroxyl oxygen atoms.

hydrogen bonds to form a distorted hexagon, alternate molecules lying on opposite sides of its plane. Two such sextets are stacked in each *c* axis repeat and their bulkier parts interlock to form cages. The major differences between the two structures (marked contractions in *c* and in therefore the length of the cavity) are the result of a change in the interlocking pattern which is directly attributable to the introduction of the methyl group in II. Minor changes are: shorter O...O distances in II (2.766(12) compared with 3.035(8) Å) and a more puckered

Fig. 2 Section through the van der Waals' surface of the cavity for I (*A*) and II (*B*).



ring of oxygens (displacements from the mean plane of +0.34 Å (compare 0.22 Å in I)); and the introduction of distortion into the half-chair heterocyclic ring. In I the ring *gem*-dimethyl carbon and the methylene carbon are almost symmetrically displaced (0.38 and 0.34 Å) on opposite sides of the ring, whereas in II the distances are 0.23 and 0.45 Å.

The marked change in cavity shape is most readily appreciated by comparing their van der Waals' surfaces. Thus, Fig. 2*a* shows that the cavity in I has a pronounced waist at  $Z = \frac{1}{2}$  which is formed by six *gem*-dimethyl groups protruding into the cage. Figure 2*b*, on the other hand, shows the complete elimination of this waist in II—indeed the central section of this cavity is the widest: the 'hour-glass' surface of I is converted into the 'chinese-lantern' shape of II.

Marked variations in clathrating specificity and in crystallographic data have been reported<sup>5</sup> in a range of specially made chemical variants of the inorganic  $M(SCN)_2(R\text{-pyridine})_4$  clathrates ( $M = Fe, Ni, Co$ ;  $R = Me, Et, vinyl$ , and so on). Another deliberate modification of an organic clathrate cage was recently reported<sup>6</sup> by Emert and Breslow who succeeded in introducing a hydrophobic 'floor' into the cavity of  $\beta$ -cyclodextrin, but the alteration in the cavity reported above is much more general and drastic. This report, therefore, provides the first detailed analysis of the changes produced by a piece of planned clathrate engineering.

DAVID D. MACNICOL  
ANDREW D. U. HARDY  
JOSEPH J. MCKENDRICK

Chemistry Department  
The University  
Glasgow G12 8QQ, UK

Received April 16; accepted May 28, 1975.

- Hardy, A. D. U., MacNicol, D. D., and Wilson, D. R., *Chem. Commun.*, 783-784 (1974).
- Hardy, A. D. U., McKendrick, J. J., and MacNicol, D. D., *Chem. Commun.*, 972-973 (1974).
- MacNicol, D. D., and Wilson, F. B., *Chem. Commun.*, 786-787 (1971).
- International Tables for X-ray Crystallography*, I (edit. by Macgillivray, C. H., and Rieck, G. D.; gen. ed. Lonsdale, K.), 253 (Kynoch, Birmingham, 1965).
- Bhatnagar, V. M., *Clathrate Compounds*, ch. 4 (Chemical Publishing, New York, 1970).
- Emert, J., and Breslow, R., *J. Am. chem. Soc.*, 97, 670-672 (1975).

## Erratum

In the article "Depths of origin of Kenyan basalts and implications for the Gregory Rift" by G. G. Goles and implications for the Gregory Rift" by G. G. Goles (*Nature*, 255, 391; 1975) the received date is incorrect. It should read Received February 24; accepted April 9, 1975.



# matters arising

## Superfoetation in fishes and the cost of reproduction

THIBAULT<sup>1</sup> has examined the effects of juvenile mortality on reproduction of normal and superfoetatus females of the family Poeciliidae and concluded that superfoetation conferred no selective advantage in an unpredictable environment in which mortality was catastrophic. A life-table model for the evolution of superfoetation shows this to be wrong.

The net reproductive rate of a population ( $R_0$ ) summarises the interaction between survivorship and fecundity. In general,

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (1)$$

where  $l_x$  is age-specific survivorship and  $m_x$  is age specific fecundity. A consequence of superfoetation is that females produce fewer young more often than usual. For example, *Poecilia reticulata* females 30–35 mm long produce about 24 young every 21 d (ref. 1). They are not superfoetatus. By contrast, *Poeciliopsis lucida* and *P. monacha* are superfoetatus and females 30–35 mm long produce about 11 young every 11 d (ref. 1). A more general comparison of superfoetatus and normal life histories is given in Table 1.

The net reproductive rate of a superfoetatus female producing young twice as often as a normal female is:

$$R_0 = m_x B_s (1 + p_s + p_s^2 + \dots) \quad (2)$$

Here,  $m_x$  is the number of young produced in interval  $x$ ,  $B_s$  is survivorship to the age of first reproduction for superfoetatus females, and  $p_s$  is survivorship to subsequent reproductive intervals. Equation (2) can be simplified<sup>2,3</sup>

$$R_0 = m_x B_s / (1 - p_s) \quad (3)$$

Similarly, the net reproductive rate of a normal female is:

$$R_0 = n_y B (1 + p + p^2 + \dots) \quad (4)$$

Where  $n_y$  is brood size,  $B$  is survivorship of a normal female to reproductive

age, and  $p$  is survivorship to subsequent ( $x$ ) intervals (Table 1). Equation (4) can be rewritten

$$R_0 = \frac{n_y B}{(1 - p^2)} \quad (5)$$

We can define the circumstances in which superfoetation and normal patterns of reproduction have equal net reproductive rates, that is, when

$$m_x B_s / (1 - p_s) = n_y B / (1 - p^2) \quad (6)$$

or when

$$p = \sqrt{\{1 - [(n_y B / m_x B_s)(1 - p_s)]\}} \quad (7)$$

Whether or not superfoetation is advantageous depends on survivorship to age at first reproduction, subsequent survivorship, and the degree to which brood size is depressed in superfoetatus females. Our example comparing *P. reticulata* with *P. lucida* and *P. monacha* is illustrative. The species are of similar size when they first reproduce. Therefore, we assume equal survival to age at first reproduction. Brood size for the superfoetatus species is half that of the normal female<sup>1</sup>, so  $m_x = 1$  and  $n_y = 2$ . Thus equation (7) becomes

$$p = \sqrt{(2p_s - 1)} \quad (8)$$

The difference in adult survivorship ( $D$ ) when net reproductive rates are identical for both strategies is defined as

$$D = \sqrt{(2p_s - 1)} - p \quad (9)$$

$D$  increases as adult survivorship declines (Fig. 1). When the probability of a normal female surviving to produce another brood (11 d in our example<sup>1</sup>) is less than 50%, superfoetation cannot evolve. When survivorship is in excess of 80%, superfoetation results in a higher net reproductive rate if it increases adult survival as little as

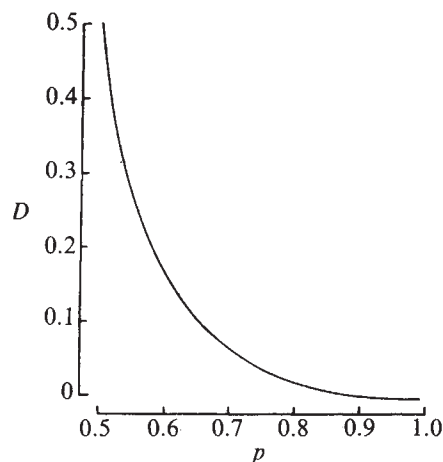


Fig. 1 Relationship between adult survivorship ( $p$ ) of normal females and the minimum increase in survivorship ( $D$ ) for superfoetation to be a more advantageous reproductive strategy.

2.5%. *P. lucida* and *P. monacha* occur in unstable environments and invade flood expanded habitats<sup>1</sup> possibly characterised by low competition and predation and, consequently, by high adult survival. As superfoetation will reduce the peak cost of reproduction and further increase adult survival, we interpret it as an adaptation that increases reproduction in transient environments.

JERRY F. DOWNHOWER  
LUTHER BROWN

Department of Zoology,  
Ohio State University,  
Columbus, Ohio 43210

<sup>1</sup> Thibault, R., *Nature*, 251, 138–140 (1974).

<sup>2</sup> Mertz, D., *Am. Nat.*, 105, 437–454 (1971).

<sup>3</sup> Goodman, D., *Am. Nat.*, 180, 247–268 (1974).

THIBAULT REPLIES — Downhower and Brown<sup>1</sup> contend that natural selection will favour fishes with superfoetation over

Table 1 Basic life table for superfoetatus and normal species

| Reproductive event | $m_x$ | Superfoetatus<br>$l_x$ | $n_y$ | Normal<br>$l_x$ |
|--------------------|-------|------------------------|-------|-----------------|
| Birth              | 0     | 1.00                   | 0     | 1.00            |
| ↓                  |       |                        |       |                 |
| First reproduction | $m_1$ | $B_s$                  | $n_1$ | $B$             |
| 2                  | $m_2$ | $B_s p_s^1$            | 0     | $B p^1$         |
| 3                  | $m_3$ | $B_s p_s^2$            | $n_2$ | $B p^2$         |
| ↓                  |       |                        |       |                 |
| $i$                | $m_i$ | $B_s p_s^{i-1}$        | $n_i$ | $B p^{i-1}$     |

fishes without superfoetation in transient environments characterised by low levels of predation and competition, and consequently high adult survivorship. Although the mechanics of the model seem sound, its theoretical applicability breaks down on examination of the biology of poeciliid fishes.

In the Rio del Fuerte of north-western Mexico, *Poeciliopsis monacha* inhabits small pools in headwater arroyos carved in solid bedrock. The raging torrents of the rainy season revert in the dry season to congested and isolated pools of a few litres of water. Fishes become compacted, are heavily parasitised and appear emaciated. Aquatic arthropod and reptilian predators are abundant, imposing

and J. Schultz, unpublished). Superfoetation does not seem to be an adaptation solely to transient environments; levels of superfoetation, instead, seem correlated with increased stability and decreased levels of competition. Moreover, approximately 85% of the species of poeciliids lack superfoetation which further attests to the flexibility of the more common and more widely distributed reproductive mechanism.

Further, there is no reason to assume that superfoetation reduces the peak cost of reproduction. Some *Poeciliopsis* have evolved high degrees of maternal contributions to developing embryos with pseudoplacentas (R.T. and J.S., unpublished). The maintenance of several

lomas. Further studies in our laboratory and in others showed that these conclusions were not correct.

The new data showing our misinterpretation were as follows. First, when TdT activity is determined in partially purified extracts from thymus, the highest activity is observed in the presence of oligo (dA) as primer, and of dGTP as substrate. When myeloma extracts were used, no activity was detected with this combination. In our previous study, the TdT activity was determined using d(pT)<sub>4</sub> with dATP or dTTP; this system seems to have very low efficiency. Second, further studies of the profile of the activities on the phosphocellulose chromatograms with thymus extracts showed an overlapping, but not an exact correspondence of the peaks obtained with oligo (dA)/dGTP and with d(pT)<sub>4</sub>/dATP. The d(pT)<sub>4</sub>/dATP activity was not modified in the thymus after hydrocortisone treatment of mice. It was recently shown that TdT is drastically decreased in these conditions<sup>2,3</sup>.

Therefore, the activity we described<sup>1</sup> does not correspond to terminal deoxynucleotidyltransferase, but to another deoxynucleotide-polymerising enzyme, the nature of which remains to be determined.

We thank D. Baltimore, R. MacCaffrey, T. A. Harrison, P. C. Kung and A. E. Silvertone for help in the analysis of myeloma and thymus activities which led us to the correct interpretation of our results.

CLAUDE PENIT  
ALAIN PARAF  
FRANÇOIS CHAPEVILLE

*Institut de Biologie Moléculaire,  
Paris, France*

<sup>1</sup> Penit, C., Paraf, A., and Chapeville, F., *Nature*, **249**, 755-757 (1974).

<sup>2</sup> Kung, P. C., Silverstone, A. E., McCaffrey, R. P., and Baltimore, D., *J. exp. Med.*, **141**, 855-865 (1975).

<sup>3</sup> Bollum, F. J., *Fedn Proc.*, **34**, 494 (1975).

## Double helix of tropomyosin

LONGLEY<sup>1</sup> has suggested that the supercoil pitch length (*P*) of tropomyosin (TM) may be 114 Å. The arguments in favour of this value are based on the assumptions that TM molecules are optimally packed in the tactoids formed using Mg<sup>2+</sup> and Ca<sup>2+</sup> (ref. 2) and also that TM presents an axially and azimuthally pseudo-equivalent aspect to consecutive actin molecules.

Although it is known<sup>3,4</sup> that two-stranded ropes may pack optimally when there is a relative axial stagger of *P*/4, it remains to be shown that TM packs in this way in Mg tactoids. Indeed, the Mg

predation pressure on all size classes of *Poeciliopsis*<sup>2</sup>. Conjecture that these environments are characterised by low levels of intraspecific competition and predation and high adult survivorship, is unfounded.

Downstream from headwater environments, the Fuerte broadens and is somewhat stable with a diversity of lentic and lotic habitats. Inhabited by *P. lucida*, these tributaries are home to an equally diverse array of aquatic predators as well as avian predators. The robustness of fishes in this habitat reflects reduced levels of competition. These tributaries drain into the main stems of the Fuerte with deep and flowing permanent water and stable temperature regimes. Superfoetated *P. prolifica* is found in these environments.

In terms of stability, stream size, and competition levels, the habitat of *P. monacha* lies at one extreme, being transient with competition pronounced. The environment of *P. prolifica*, in contrast, is stable with low levels of competition. The environment of *P. lucida* is intermediate in these characteristics. Predation pressure is variable among the environments. Populations of reproducing *P. monacha* average 1.4-1.5 broods per female developing simultaneously; *P. lucida*, 1.8-2.0; and *P. prolifica* 4-5 (R.T.

broods, each with specific nutritive and metabolic demands, may instead enhance the cost of reproduction.

In extending a hypothetical life table (Table 1) to enable calculation of *r* (the intrinsic rate of increase<sup>3</sup>), fishes without superfoetation have a slight advantage. Regardless of female survivorship, the ability to produce a large number of young in the initial reproductive period may be adaptive in transient environments where *r* selection is operative.

Superfoetation must convey some evolutionary advantages to viviparous fishes; however, the causative selective force, to me, still remains an enigma.

*Bowling Green State University.*

<sup>1</sup> Downhower, J., and Brown, L., *Nature*, **256**, 345 (1975).

<sup>2</sup> Thibault, R., thesis, Univ. Conn. (1974).

<sup>3</sup> Birch, L., *J. Anim. Ecol.*, **17**, 15-26 (1948).

## Terminal deoxynucleotidyltransferase in murine myelomas

WE have published<sup>1</sup> results suggesting the presence of terminal deoxynucleotidyltransferase (TdT) in murine mye-

**Table 1** Calculation of *r* for populations of fishes with and without superfoetation

| (10 d interval)        | With superfoetation  |                                                 | Without superfoetation |                                                 |
|------------------------|----------------------|-------------------------------------------------|------------------------|-------------------------------------------------|
|                        | <i>m<sub>x</sub></i> | <i>l<sub>x</sub></i>                            | <i>m<sub>x</sub></i>   | <i>l<sub>x</sub></i>                            |
| 1                      | 0                    | 1.00                                            | 0                      | 1.00                                            |
| ↓                      | ↓                    | ↓                                               | ↓                      | ↓                                               |
| 8 (First reproduction) | 10                   | <i>p</i> - <i>n</i> <sub>1</sub> (0.025)        | 20                     | <i>p</i> - <i>n</i> <sub>1</sub> (0.025)        |
| 9                      | 10                   | <i>p</i> - <i>n</i> <sub>2</sub> (0.025)        | 0                      | —                                               |
| 10                     | 10                   | ↓                                               | 20                     | <i>p</i> - <i>n</i> <sub>3</sub> (0.025)        |
| 11                     | 10                   | ↓                                               | 0                      | —                                               |
| 12                     | 10                   | ↓                                               | 20                     | ↓                                               |
| 13                     | 10                   | ↓                                               | 0                      | —                                               |
| 14                     | 10                   | ↓                                               | 20                     | ↓                                               |
| ↓                      | ↓                    | ↓                                               | ↓                      | ↓                                               |
| <i>i</i>               | <i>m<sub>i</sub></i> | <i>p</i> - <i>n</i> <sub><i>j</i></sub> (0.025) |                        | <i>p</i> - <i>n</i> <sub><i>j</i></sub> (0.025) |

*n*, Integer values from 0 to *j*.

As *p* → 0.8 (high survivorship), *r* → 0.37

*r* → 0.41.

As *p* → 0.3 (low survivorship), *r* → 0.32

*r* → 0.37.

*l<sub>x</sub>*, age-specific survivorship; *m<sub>x</sub>*, age-specific fecundity; *p*, survivorship adjustment factor to determine survivorship at each interval.



tactoid geometry may simply be explained as follows: the distribution of acidic residues of a TM chain has approximate mirror symmetry about a point  $\sim 70\text{\AA}$  from the N terminus. The assembly of the tactoids may not be controlled solely from packing considerations, but may be dominated by the bridging effect of  $\text{Mg}^{2+}$  between acidic moieties of oppositely directed molecules. If TM molecules were optimally packed in the positively and negatively stained tactoids, there is still no *a priori* reason to believe that  $P/4$  should be equated to the observed  $28\text{\AA}$  axial period.

Even with an axial pseudo-period of about 19.5 residues in acidic and in apolar residues not in series I and II (refs 5 and 6), azimuthally-identical pseudo-equivalent actin-TM interactions could only occur if an actin separation along one strand ( $\sim 2 \times 19.5$  residues) corresponds to a multiple of  $P'$  (unless the two chains of TM are homologous and in axial register, in which case actin separations could correspond to a multiple of  $P'/2$ ).  $P'$  is the supercoil pitch length measured along the helical axis of the supercoil. On packing grounds, it has been suggested that the two chains of TM may have a relative axial stagger of 14 residues<sup>6-8</sup> thus facilitating head-to-tail assembly of TM molecules, but recent evidence has cast doubts on this conclusion<sup>9,10</sup>.

Irrespective of the relative chain stagger in TM, the length and flexibility of side chains may enable the first and/or second chain of TM to make pseudo-equivalent interactions with an actin strand even if precise azimuthal relationships are not maintained (see, for example, Hulmes *et al.*<sup>11</sup> for collagen).

Theoretically, the pitch length of  $\alpha$ -fibrous proteins may be found directly using a measurement of the axial separation of the equatorial and near equatorial reflections in the X-ray diffraction patterns<sup>12</sup>. Inherent accuracy, however, is usually low as a result of partial overlapping of the relevant data. Also the possibility that axial sampling moves the maxima of the molecular transform cannot be completely eliminated<sup>13</sup>.  $P$  has been estimated for  $\alpha$  keratin<sup>14</sup> (140–170  $\text{\AA}$ ); paramyosin<sup>4,15</sup> (136–140  $\text{\AA}$  and 178  $\text{\AA}$ ) and honeybee silk<sup>16</sup> (140  $\text{\AA}$ ), but not for TM. If the head-to-tail assembly of TM molecules in the grooves of the thin filament form a regular supercoil and troponin has an invariable azimuthal relationship with TM and actin, then  $nP = 410 \pm 4\text{\AA}$  (ref. 17) where  $n$  is integral. If  $n = 3$ , then  $P = 137\text{\AA}$  and there would be six half-turns of supercoil associated with seven actin molecules. As McLachlan and Stewart (private communication) have also pointed out, TM would then present pseudo-equivalent aspects to all seven actins with which it makes contact, as  $3P = 7P'/2 = 410\text{\AA}$  where  $P'/2 \sim 2 \times 19.5$  residue translations

of an  $\alpha$  helix or, equivalently, an actin separation.

The pitch length of an  $\alpha$ -fibrous coiled coil cannot be continuously reduced without a concomitant increase in the deformation of bond lengths and angles. The point at which the coiled coil structure reaches a minimum energy state is not known, although it is tempting to speculate that this may occur when  $P$  is about 140  $\text{\AA}$ , a value experimentally noted for several  $\alpha$ -fibrous proteins.

D. A. D. PARRY

Department of Chemistry, Biochemistry  
and Biophysics,  
Massey University,  
Palmerston North, New Zealand

- <sup>1</sup> Longley, W., *Nature*, **253**, 126–127 (1975).
- <sup>2</sup> Cohen, C., and Longley, W., *Science*, **152**, 794–796 (1966).
- <sup>3</sup> Rudall, K. M., *Lectures on the Scientific Basis of Medicine*, V, 217–230 (Athlone, London, 1956).
- <sup>4</sup> Elliott, A., Lowy, J., Parry, D. A. D., and Vibert, P. J., *Nature*, **218**, 656–659 (1968).
- <sup>5</sup> Miller, A., *M.T.P. Reviews of Biochemistry* (edit. by Blasco, H.), (Butterworths, London, in the press).
- <sup>6</sup> Parry, D. A. D., *Biochem. biophys. Res. Commun.*, **57**, 216–224 (1974).
- <sup>7</sup> Hodges, R. S., Sodek, J., Smillie, L. B., and Jurasek, L., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 299–310 (1972).
- <sup>8</sup> Stone, D., Sodek, J., Johnson, P., and Smillie, L. B., *Proc. ninth FEBS meeting, Budapest* (in the press).
- <sup>9</sup> Johnson, P., and Smillie, L. B., *Biochem. biophys. Res. Commun.* (in the press).
- <sup>10</sup> Stewart, M., *FEBS Lett.*, **53**, 5–7 (1975).
- <sup>11</sup> Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A., and Woodhead-Galloway, J., *J. molec. Biol.*, **79**, 137–148 (1973).
- <sup>12</sup> Crick, F. H. C., *Acta Cryst.*, **6**, 689–697 (1953).
- <sup>13</sup> Fraser, R. D. B., and MacRae, T. P., *Conformation in Fibrous Proteins and Related Synthetic Polypeptides* (Academic, New York and London, 1973).
- <sup>14</sup> Fraser, R. D. B., *et al.*, *Appl. Polymer Symp.*, no. 18, 65–83 (1971).
- <sup>15</sup> Cohen, C., and Holmes, K. C., *J. molec. Biol.*, **6**, 423–432 (1963).
- <sup>16</sup> Atkins, E. D. T., *J. molec. Biol.*, **24**, 139–141 (1967).
- <sup>17</sup> Cohen, C., Caspar, D. L. D., Parry, D. A. D., and Lucas, R. M., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 205–216 (1971).

LONGLEY REPLIES—In calculating an example of his model of the coiled coil, Crick<sup>1</sup> started with a straight  $\alpha$  helix having 18 units in 5 turns, giving a coiled coil with 126 residues in a pitch of 186  $\text{\AA}$ . If, instead one starts with the very similar helix of 11 units in 3 turns, the resulting coiled coil has 77 residues in a pitch of 114  $\text{\AA}$ . (The 11/3 helix is one of the alternatives considered by Pauling and Corey<sup>2</sup> and is the structure automatically formed when an  $\alpha$  helix is constructed of CPK molecular models.) Thus, large variations in the pitch of a double helix can result from small changes in the  $\alpha$  helix from which it is derived, and there is no reason why the pitch of tropomyosin (TM) should be the same as that of paramyosin, keratin or bee silk. The main resistance to bending the  $\alpha$  helix into a tighter coiled coil is in stretching and compressing the H bonds<sup>3</sup>. To bend it into a helix of pitch 114  $\text{\AA}$  would require 0.1–0.2 kcalorie  $\text{mol}^{-1}$  which is not large, and would strain the H bonds by only 2 or 3%. The observed spread in H-bond lengths is about 8% (ref. 4).

Now, 77 is an odd number so that each

alternate half-turn of such a double  $\alpha$  helix would have a different pattern of side groups from the next. This, together with the 14-residue stagger, could be accommodated in the thin filament of muscle if the pattern of contacts between TM and actin alternated (and perhaps commutated) along the length of the TM molecules. In the 'tactoids' likewise, cross linking (by divalent cations) would be simpler if the chemical repeat were compatible with the helix pitch.

Duke University Medical Center

- <sup>1</sup> Crick, F. H. C., *Acta Cryst.*, **6**, 689–697 (1953).
- <sup>2</sup> Pauling, L., and Corey, R. B., *Fortschr. Chem. org. NatStoffe*, **11**, 180–239 (1954).
- <sup>3</sup> Crick, F. H. C., *Nature*, **170**, 882–883 (1952).
- <sup>4</sup> Ramakrishnan, C., and Prasad, N., *Int. J. Protein Res.*, **3**, 209–231 (1971).

## Number counts of $\gamma$ -ray bursts

STRONG and Klebesadel<sup>1</sup> suggest that most of the 23  $\gamma$ -ray bursts detected by the Vela satellites are of galactic origin. I question their arguments, which are based on the distribution in galactic latitude and longitude of those bursts for which coordinates have been determined, as well as on the source counts. They admit that the latitude distribution is not significantly galactic. Similarly, the longitude distribution is of low significance. If, *a priori*, the local spiral arm had been predicted to dominate this distribution, then the observations have a chance probability of  $\sim 1.5\%$ . This probability rises above 6% if the observations in any way suggest the correlation. Further, the location of the event of April 27, 1972 (ref. 2) (which was not used in the reported analysis) does not easily fit into the simple local spiral arm hypothesis.

The source counts as presented give the number of events of time-integrated flux density,  $S$ , exceeding some level  $S_0$ . As  $S_0$  decreases, the source counts flatten out below the three-halves dependence expected from a uniform source distribution. This seems to indicate a scarcity of weak sources, which is consistent with a galactic origin for the bursts. Such a flattening could perhaps also be caused by the first trigger of the event as well as by the method by which the end of the event is determined. The first trigger effects have been partially eliminated by Strong and Klebesadel and it is evident from their figure caption that the second has been noted, although it is not discussed in the text.

I consider this effect in more detail and assume an abrupt rise and gradual fall-off for the burst represented by:

$$\begin{aligned} I &= 0 \\ I &= Ke^{-t/\tau} \end{aligned} \quad \left. \begin{array}{l} t < 0 \\ t \geq 0 \end{array} \right\}$$

The time-integrated flux  $S$  is given by

$$S = K\tau(1 - e^{-t/\tau})$$

where  $t$  is the apparent duration of the burst. This will be determined by the detector noise level, which I assume to be constant and to imply a lowest sensitivity of  $L \text{ erg cm}^{-2} \text{ s}^{-1}$ . Now  $t = \tau \ln(K/L)$ , therefore  $S = (K - L)\tau$ .

If the sources are distributed uniformly,

$$N(>K) = CK^{-3/2}$$

$$\text{and then, } N(>S) = \tau^{-2}C(S/\tau + L)^{-3/2}$$

or

$$N(>S) \propto (S + L\tau)^{-3/2} \quad (1)$$

So when  $S \gg L\tau$ , a three-halves dependence is expected, but when  $S \ll L\tau$ ,  $N(>S)$  is independent of  $S$ . This is because the events rapidly seem to weaken as  $K$  approaches  $L$ . The dependence given by equation (1) is of a reasonable form to fit the source counts and implies

$$L\tau \approx 5 \times 10^{-6} \text{ erg cm}^{-2}$$

$\tau$  is not necessarily a constant from event to event and is only representative of the time scale of event decay.

I conclude that the arguments for a galactic origin for  $\gamma$ -ray bursts are by no means sound, and that the distance estimates are extremely uncertain. Number counts of  $\gamma$ -ray bursts, or any other transient phenomena, of time-integrated fluxes less than about  $2L\tau$  may merely reflect the average time structure of the events rather than inhomogeneities in source distribution. Many more observations over a much wider range of sensitivity are required if source distances are to be inferred from the number counts. Sensitive  $\gamma$ -ray detectors carried whenever possible in satellite, space-probes or long-duration balloon flights would be invaluable in this respect.

I thank the SRC for support.

A. C. FABIAN

*Institute of Astronomy,  
Cambridge CB3 0HA, UK*

<sup>1</sup> Strong, I. B., and Klebesadel, R. W., *Nature*, **251**, 396-397, (1974).

<sup>2</sup> Trombka, J. I., et al., *Astrophys. J. Lett.*, **194**, L27-L33 (1974).

**STRONG AND KLEBESADEL REPLY—**  
The arguments used in our letter<sup>1</sup> may be questioned on several grounds, but not those considered by Fabian<sup>2</sup>, who makes two principal assumptions. These are the flux as a function of time may be

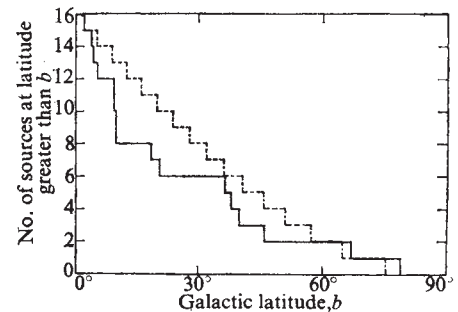
represented adequately by a form having an abrupt rise to maximum followed by exponential decay, and the apparent duration of the bursts is limited by the detector noise level. Given that these were valid we would agree with his analysis. But both assumptions, although seemingly reasonable, are incorrect. The event of April 27, 1972 is an excellent counter-example<sup>3,4</sup>. Our paper did not include this case as it was written before the papers by Metzger *et al.*<sup>3</sup> and Trombka *et al.*<sup>4</sup> were published. Further examples may be found in ref. 5.

The actual time profiles of  $\gamma$ -ray bursts are very variable, ranging from single, very intense spikes to complex structures with one or more precursor pulses, a main burst comprising a number of sub-bursts lasting about a second each, and with pronounced substructure, and often a resurgence of weaker, but similar activity a few seconds to a minute later. If a decaying exponential were even an approximately correct model one would expect a strong correlation between  $S$  and the apparent duration,  $t$ . For values greater than  $S = 3 \times 10^{-6} \text{ erg cm}^{-2}$ , where we have noted that we expect trigger-threshold effects, this is not the case<sup>6</sup>. The Vela detectors can provide reliable values of  $S$  well below those of most of the measured events, if we assume the time profiles are similar to the stronger ones. We do not record these events because they fail to trigger the system. In other words, in dealing with low values of  $S$  we are trigger-limited rather than limited by the detector noise level.

Although Fabian's detailed argument is therefore not relevant this does not mean that we are still satisfied with our interpretation of the log  $N$ -log  $S$  plot. We had assumed, like Fabian, that the system would trigger only near the start of an event, and that trigger-threshold effects would appear as an increasing trigger-failure rate with weaker signals. Our threshold figure of  $3 \times 10^{-6} \text{ erg cm}^{-2}$  meant that we would fail to detect a significant fraction of events with measured  $S \lesssim 3 \times 10^{-6} \text{ erg cm}^{-2}$ . What can happen is that even for  $S > 3 \times 10^{-6} \text{ erg cm}^{-2}$  the instantaneous flux may be low and a trigger, if it occurs at all, can take place after the start of the event. The event<sup>3</sup> of April 27, 1972 illustrates this. The Vela 6A system recorded only the last third of the event, giving  $3 \times 10^{-6} \text{ erg cm}^{-2}$  instead of  $\sim 10^{-4} \text{ erg cm}^{-2}$ . Putting it simply, we had considered only failures to trigger which gives rise to errors in  $N$ . We did not consider errors in  $S$  caused by late triggering. The range over which the log  $N$ -log  $S$  relationship is reliable therefore lies at  $S > 10^{-4} \text{ erg cm}^{-2}$ . This contains too few events to be significant. (We note that the reported values of  $S$  from Vela data<sup>6</sup>, and those used here, are low by a factor of about two compared with accurate

simultaneous measurements made on some other spacecraft, and are probably all underestimated by this amount.)

Turning to the directional distributions, Fabian agrees with our assessment of their significance as being low. They are not therefore to be ignored. The point of our letter was to draw attention to the possibility that the data contained information relevant to the problem of determining the source distances, and not that there was conclusive evidence for a galactic distribution. We also intended to show how future data could be incorporated for this purpose. Work carried



**Fig. 1** Integral galactic latitude distribution for 16 gamma-ray burst sources. —, Observed distribution; ---, isotropic distribution.

out by many people over the past year has provided additional source directions. This has had the effect of largely removing the original anisotropy in longitude, and of further emphasising the preference for low latitudes (Fig. 1). We note that two of the high-latitude sources have large errors ( $\sim \pm 15^\circ$ ) and that one additional source on the Galactic Equator should probably be added.

This is again suggestive of a galactic association, but we accept the possibility that in a few cases we may be witnessing repeats from the same source. If this turns out to be true it is extremely important, but would mean that the distribution of sources as opposed to events would follow more closely the curve for an isotropic distribution.

This work was supported by USERDA. We thank T. Gold, A. Treves, L. Maraschi and others for discussions on the interpretation of the size spectrum which have led to our reconsideration. We were too late to mention this in ref. 1, but have added a note in the reprints.

<sup>1</sup> Strong, I. B., and Klebesadel, R. W., *Nature*, **251**, 396-397 (1974).

<sup>2</sup> Fabian, A. C., *Nature*, **256**, 347 (1975).

<sup>3</sup> Metzger, A. E., Parker, R. H., Gilman, D., Peterson, L. E., and Trombka, J. I., *Astrophys. J. Lett.*, **194**, L19-L25 (1974).

<sup>4</sup> Trombka, J. I., et al., *Astrophys. J. Lett.*, **194**, L27-L33 (1974).

<sup>5</sup> Strong, I. B., *Proc. ESLAB Symp. Context and Status of  $\gamma$ -Ray Astronomy* (ESRO, in the press).

<sup>6</sup> Strong, I. B., and Klebesadel, R. A., *Astrophys. J. Lett.*, **188**, L1-L3 (1974).

<sup>7</sup> Strong, I. B., Klebesadel, R. A., and Evans, W. D., *Ann. NY Acad. Sci.* (in the press).



# reviews

THE value and dangers of drawing analogies between organism and societies have been debated at least since Herbert Spencer; part of their appeal is that they seem to provide a scientific language for talking about society and social organisation, which are prickly subjects of which scientists and doctors usually know little. Dr Salk is open and unashamed about it: "I believe in the usefulness of biological analogies in thinking about men." He calls it "a theoretical-experimental way of thought", to be distinguished from "a 'philosophical speculative way of thought' for dealing with questions in the human realm." He doesn't think much of philosophers, and we hear no more about them, not even those who have studied these problems through the ages. Instead he provides a long series of analogies. Racial intolerance may follow laws similar to immunological intolerance, and "then patterns of possible interest in psychological phenomena, could be observed through quantitative studies in immunology". The phenomena of enzyme induction are held to show something of the environmental forces that are needed to nurture and encourage the fulfilment of our potentialities. He takes the analogies very seriously, but can one really hope that they will, as he suggests, help in adjusting the relationships "between parent and child, teacher and student, lovers,

## Biology and man

J. Z. Young

*How Like an Angel: Biology and the Nature of Man.* By Jonas Salk. Planned and edited by Ruth Nanda Anshen. Pp. 118. (David and Charles: Newton Abbot, London and Vancouver, April 1975.) £3.50.

peers, or between groups or nations?"

The general aim of the book is to show that biological knowledge is increasingly relevant to human affairs, and though this is indeed true perhaps the continual stressing of analogies is not the best way to make the point. Those who actually have to manage social, educational and political affairs are not very likely to pay attention to such generalities, and will ask for more concrete examples. Dr Salk is continually emphasising that we "must" change our ways. "What is needed is a change of perspective . . . new values and new ethics are required . . . it will be necessary to establish patterns in the young child that will help him to develop to his fullest." He does not show any understanding that questions as to how these things can be done have occupied men for centuries and are at present being studied intensively.

Those who work in such fields may be annoyed to read that "a beginning must somehow be made". Perhaps more sociology for medical students might be a good start.

In view of Dr Salk's own contributions to infantile paralysis it is natural to look especially carefully at the social analogies he draws with this disease. He indeed believes that there is "a new form of crippling": Man's mind, which has "developed dinosaurian qualities (and) threatens to overpower his body", now apparently "faces uselessness". And our "socially and economically advantaged youths", because of their easy upbringing, are not immunised against this new disorder in the way that they have been immunised against polio. It is not clear whether the conclusion from this analogy is that we should inflict greater hardships upon children of the rich to immunise them against this disease.

It would be unfair to ridicule all of Salk's analogies. He is well up-to-date with studies of the development of the brain and its need for stimulation at appropriate stages of development. Not unexpectedly, he draws an analogy between learning and selective theories of antibody formation. But this is more than an analogy, and it is disappointing that he does not tell us about the evidence that the brain does actually begin with a library of possibilities from which selection is made. □

PROFESSOR LURIA has produced a very interesting book from the edited transcript of the general biology course he taught at MIT. Its theme is the central place of the genetic programme in biology. In my opinion he succeeds very well in presenting his material (primarily on cell biology) in a logical self-sustaining and interesting manner. Although he provides references to other books he accommodates within his own the essential argument and examples. There are concise appendices on chemical topics such as kinetics, free energy and the structures of biological molecules, and other appendices on discussion topics and examination questions.

The first part of the book (four lecture-chapters) introduces the characteristics of living matter and gives a good account of the cellular components. The section on biochemistry

(nine lectures) proceeds from enzymes through energetics and the generation of ATP to the biosynthesis of amino acids, nucleic acids and proteins, but not of carbohydrates and lipids. That on genetics (eight lectures) starts with

*Lectures in Biology.* By S. E. Luria. Pp. xvii+439. (MIT Press: Cambridge, Massachusetts, and London, 1975.) n.p.

Mendel, and deals, in order, with cell cycles and life cycles, bacterial and phage genetics, eukaryotic genetics (almost entirely *Drosophila* and Man) and population genetics and evolution. An excellent feature here, and indeed throughout the book, is the way in which Luria demonstrates the value of particular systems in illuminating particular problems without making his treatment seem fragmented. Thus, in the section on developmental biology

(seven lectures) bacterial sporulation, slime moulds, plant gametogenesis and meristems, insects, sponges, *Hydra*, snails, *Amphioxus*, chickens and mice are all used as illustrations. The entry into the discussion of (vertebrate) physiology (eight lectures) is through hormones; there are then chapters on muscle, blood and ionic balance, immunity and neurobiology.

Given the area covered, it is not surprising that the treatment is dogmatic, and that the amount of attention given to how actual experiments were done (and who did them) is generally scant. This is an excellent book for those seeking an exposition of how living systems function. It will be particularly valuable for the more junior university student, physical scientists in search of biology and teachers of biology wanting to keep abreast of cell biology. **Paul Broda**

## Elementary particle theories and phenomena

*Particle Interactions at Very High Energies.* Edited by David Speiser, Francis Halzen and Jaques Weyers. Part A: Pp. xii+398. \$33.60. Part B: Pp. xiii+366. \$30.00. (NATO Advanced Study Institutes Series.) (Plenum: London, 1974.)

THESE two volumes cover the main lectures given at the Summer Institute on Elementary Particle Physics held at Louvain in August 1973. Looking back over the years it is probably fair to say that almost every summer school volume that has appeared has contained at least one noteworthy set of lectures, and the present volumes are no exception. Although both books shelter under the umbrella of general elementary particle physics, they are really very different in content and subject matter, and it is, therefore, best to deal with them separately.

Part A is concerned with hadronic interactions at very high energies, and concentrates mainly upon the phenomenological results that have been flowing in such profusion from the CERN Intersecting Storage Rings (ISR) and,

more recently, from the Fermi National Accelerator Laboratory near Chicago. It touches, too, upon the theories and models that have been conjured up to try to interpret the vast amount of data. But if there is a single overwhelming impression left by the reading of this volume it is simply one of amazement and awe; amazement at the absolutely prodigious flow of data from the new machines and awe in the face of the fascinating and complex behaviour of nature at these energies.

There is considerable overlap between several of the lecturers. The most interesting article is Sens', which covers the shadowy area of physics lying between the domain of the high energy physicist and that of the machine engineer: namely the design and structure of the ISR, its beam characteristics, its performance level, its instabilities and their cures, and the connection between the raw measurements and the quantities of physical interest. It is particularly exciting to read of Sens' optimism about the possibility of storing antiprotons in the ISR, using 400-GeV protons from the new CERN Super Proton Synchrotron (SPS) as a source of 25-GeV antiprotons. One awaits with great expectation and some trepidation the results of proton antiproton collisions at ISR energies—many a theory will surely topple.

All three authors concentrate on proton-proton collisions at very high energies, with coverage of elastic reactions, total cross-sections and many body production, the latter in the guise of 'inclusive' reactions. Jacob's treatment of the data is detailed and very wide ranging. Horn applies himself more to "general trends", that is, to the approximate phenomenological rules that seem to summarise the data. Both Jacob and Horn discuss the theoretical background, but neither does so in a sufficiently comprehensive manner to allow the non-expert to follow. Horn does succeed, though, in giving one a taste of the remarkable success enjoyed by Regge theory, in its extension to many body reactions by Mueller, at least in explaining the gross features of the data.

Finally, there are the notes of Halzen (Model Independent Features of Diffraction) which provide a rather pedantic, and sometimes careless, list of theoretical results that can be derived from 'deep' principles, that is, from axiomatic field theory. Some discussion of their relevance, or lack of relevance, to present day experiments is given.

All in all this is an interesting, though not distinguished, collection of notes the value of which is not a little diminished by the outrageous presence of 18 blank pages.

Part B reminds one of how much easier it is to write elegantly on a purely theoretical topic than on a phenomenological one. All the lectures are

clear; arguments are logically developed; examples reinforce one's understanding, and one emerges feeling that one has been subjected to a valuable pedagogical experience.

Gilman (Deep Inelastic Scattering and Final State Hadrons) gives a good summary of the theory of electron-proton and neutrino-proton collisions at large momentum transfer, with a judicious blend of ideas about scaling, partons, the structure of the produced hadrons, and just enough of experiment to motivate the treatment. The article by Weyers (Constituent Quarks and Current Quarks) tackles one of the most tantalising problems in elementary particle theory, namely the relationship between those quarks out of which the hadrons seem to be built, and the quarks in terms of which the weak and electromagnetic currents are simply expressed. There is a useful summary of the pros and cons of the group  $U(6)$  as a classification group for the hadrons, and a good pedagogical introduction to the Melosh transformation. Carlitz (Chiral Symmetry and the Hadron Spectrum) repeats much of the material of Weyers, but less adequately. It is hard to justify the duplication. A united effort by the two authors would surely have been more successful.

The great mystery about quarks is their non-appearance as identifiable, individual particles in the real world. Models have been blithely advocated in which the quarks are permanently bound inside the hadrons which they constitute. In his article (Permanently Bound Quarks) Mandula presents a fascinating speculative study of a 'field theory' in which the fundamental quanta cannot materialise as free particles. The picture, though a little contrived does expose the basic and somewhat frightening difficulties.

The other principal article in this volume, a major *tour de force* that was originally issued as a CERN Yellow Report, is the evocatively titled "Diagrammar" of 't Hooft and Veltman. Inspired by the difficulties of applying the canonical rules of field theory to gauge theories, they have tried to reformulate all the essential rules in terms of a diagrammatic calculus (essentially a sophisticated form of Feynman's propagator approach) and to de-emphasise completely both the role of the field and the Lagrangian. Many of the derivations of standard results, for example, of the Cutkosky rules for discontinuities of Feynman diagrams, are far clearer and simpler than in the conventional treatments. The  $n$ -dimensional regularisation method invented by the authors, is also discussed in some detail. This is a long article and, generally, is very clear, but it leaves one with the feeling that just a little more effort would have ironed out the few irritating sections in which the reasoning is difficult to follow.

E. Leader

## BOOKS

### ON PURE AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

### SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £4.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

**H. K. LEWIS & Co. Ltd.**

LONDON: 136 GOWER STREET,  
WCIE 6BS

Telephone: 01-387 4282



## Growing crystals

*Crystal Growth: Theory and Techniques*, vol. 1. Edited by C. H. L. Goodman. Pp. ix+300. (Plenum: New York and London, 1974.) \$33.60.

A FEW years ago essentially only one textbook on crystal growth existed. Today we have a wide choice, particularly as the assembly of a few miscellaneous articles between hard covers frequently masquerades as a textbook. An addition to both categories might be expected to cause only a slight shudder; in fact I found the present volume to be interesting and rewarding.

The articles, ably selected and edited, cover two subjects that have received little previous attention, and two that are both topical and important. The rapid development in crystal growth in recent years has forced redundancy on some of the earlier books on the subject and the appearance of comprehensive reviews aimed at a suitably high level is not only desirable, but perhaps essential if a new generation of crystal growers is to evolve.

The first section, dealing with 'Mechanisms in Vapor Epitaxy of Semiconductors' is adequate but pedestrian. This is compensated for by the excellent content and range of the section entitled 'Principles of Vapour Growth', by E. Kaldis. His *tour-de-force* includes recent work on transport theory, practical techniques and growth data. Kaldis has assembled

much useful and interesting information which makes the purchase of the book worthwhile for this section alone.

Travelling solvent techniques are dealt with by two workers who have pioneered these processes and who have achieved some remarkable successes. Although many of the materials dealt with can be grown by other methods, the preparation of large crystals of calcite has demonstrated the possibilities of the technique for a production process. Its wide applicability makes it useful as a research tool for both bulk and thin layer single crystals.

The final article deals with refractory metal crystals. Although specialised, useful information is given on techniques which are possibly not familiar to many crystal growers; the section is worth reading by all.

The book has restored my faith in current publications. I await the appearance of further volumes in this series without trepidation.

E. A. D. White

## Reproductive aspects

*Physiology and Genetics of Reproduction*. Parts A and B. (Basic Life Sciences, vol. 4.) Edited by Elsimar M. Coutinho and Fritz Fuchs. Part A: Pp. xxi+417. Part B: Pp. ix+454. (Plenum: New York and London, 1974.) \$38.50 each.

THESE volumes report the proceedings of an International Symposium on

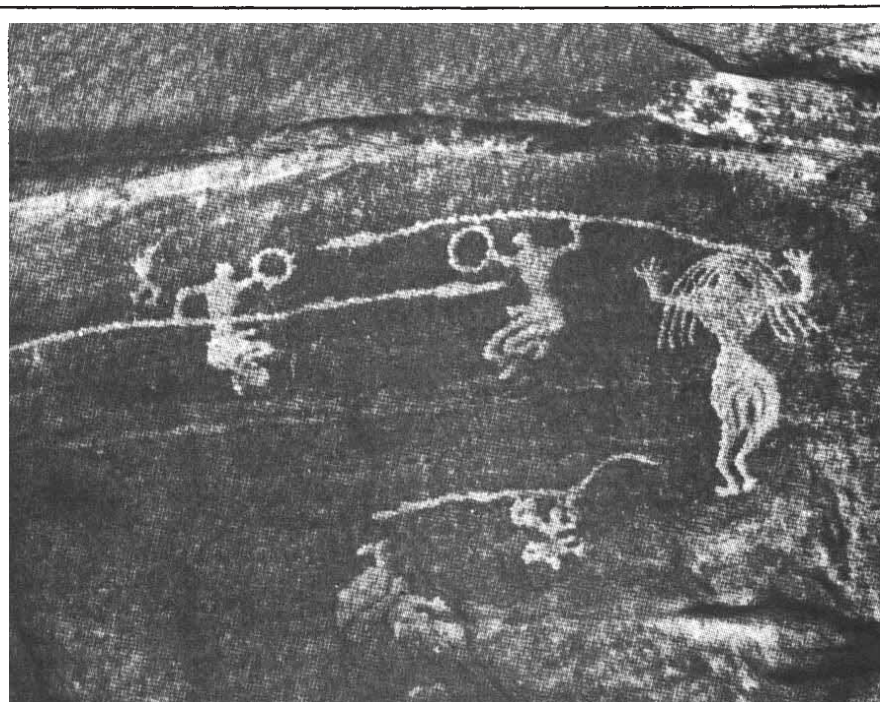
"Physiological and Genetic Aspects of Reproduction", held during December 1973 in Salvador, Brazil, to integrate knowledge of genetics and reproductive biology.

The books contain more than 50 articles covering a wide range of topics. Part A begins with a review on changes in reproductive life-span, the relationship between parity and breast cancer, and the thought-provoking concept that women in 'primitive' societies would normally have been pregnant or lactating and that, therefore, menstruation may be a 'disease' of modern society. The remainder of this volume is subdivided into four sections. The first deals with chromosomal structure and function, sex chromosome activity in germ cells, and chromosomal errors related to gonadal dysgenesis. The second section is concerned with the molecular basis of hormone action, and the third section reviews the control and inhibition of spermatogenesis, the effects of  $\gamma$  rays, and sperm structure and biochemistry. Finally, there are considerations of the role of the central nervous system; releasing hormones; protein synthesis in the egg; follicular growth, ovulation induction, luteinisation and steroidogenesis; and control of the menstrual cycle.

The second volume also contains four sections, the first of which examines structural and biochemical changes in spermatozoa, eggs and oviducts; species specificity in egg-sperm interaction; and inhibition of fertilisation with specific antibodies. Section 2 considers control of contractility in male and female reproductive ducts (ejaculation, tubal transport, uterine movements, and so on), and Section 3 reviews implantation, decidualisation, embryonic development, and chromosomal abnormalities in human abortuses. The final section discusses the control of luteal function, foetal and maternal hormones in pregnancy, uterine activity, and parturition.

The broad scope of these books ensures that they will be of interest to a wide audience, especially as a high proportion of the chapters consist of reviews. The main criticisms apply generally to conference proceedings: some topics are omitted or inadequately covered (oogenesis; contraception) whereas others are repeated in various sections (such as uterine activity). Furthermore, some of the contributions are remarkably similar to others by the same authors published elsewhere. By far the most serious criticism is that the discussions which followed each paper at the symposium have been omitted from the proceedings: such comments are sometimes more useful and thought-provoking than the articles themselves. In spite of these criticisms, however, the books provide a clear insight into a wide variety of fields.

T. G. Baker



Hunters engraved in rock. From *Rock-Art in Central Arabia*. Vol. 4: *Corpus of the Rock Engravings*, Parts III and IV. Pp. 262. (Institut Orientaliste de l'Université Catholique de Louvain: Louvain-la-Neuve, 1974.) n.p.

## Dealing with poisoned patients

*The Poisoned Patient: The Role of the Laboratory.* (Ciba Foundation Symposium 26, New Series.) Pp. viii+325. (Associated Scientific: Amsterdam, Oxford and New York, 1974.) Dfl. 55; \$21.20.

THIS volume comprises 17 papers devoted to various aspects of poisoning; each is accompanied by a very full and frequently thought-provoking discussion.

Almost one third of the papers deal with drug detection and assay techniques, such as gas chromatography linked with mass spectrometry, radio immunoassay and associated methodological problems. The controversy over the place of the laboratory in the management of patients who have taken overdoses is covered by Newton and Prescott of Edinburgh who argue spiritedly against Goulding of London and the North American contingent. My impressions are that all of the participants advocate conservative management of the majority of patients and that differences in emphasis of active treatment and demands for drug profile results would disappear if all specialists saw a similar spectrum of patients.

The least well covered aspect in the symposium concerns the role of the laboratory in detecting and preventing drug induced disease. Leach's paper on this subject is mainly concerned with refinement in existing drug assay techniques. The tantalising problem of converting present methods which are mainly concerned with single 'once off' assays into systems capable of dealing with many samples is barely touched on, and no mention is made of the claims of improvement in patient management that have followed the introduction of procedures such as the immunoassay of digoxin. It is estimated that between 5 and 10% of hospital beds are filled by patients suffering from drug-induced diseases. Presumably, that figure indicates the tip of the iceberg with respect to the total quantity of drug-induced ill health.

Dole contributes a stimulating paper on the role of the laboratory in the treatment of narcotic poisoning. In the US it is a federal requirement that the urine of those patients receiving maintenance methadone be tested weekly. That, however, has been found to be positively detrimental to the rehabilitation of patients, as it fosters non-cooperative attitudes.

Repeatedly during the discussions it is claimed that doctors are not sufficiently trained in clinical pharma-

cology, so that no matter how sophisticated and accurate are the assay techniques used, the data obtained will be wasted. This book should stimulate interest in clinicians, thus going some way towards overcoming this very real gap in medical education and helping towards the establishment of useful collaboration between clinical and laboratory workers. **Noel Wright**

## Biological interface

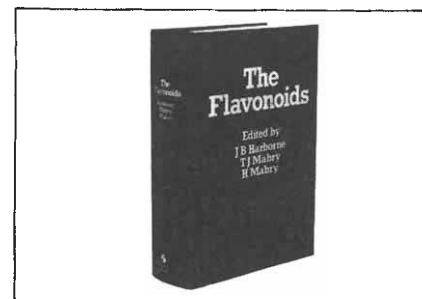
*Biological Interfaces: An Introduction to the Surface and Colloid Science of Biochemical and Biological Systems.* By Malcolm N. Jones. Pp. ix+240. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.34; \$13.25.

THERE is no question that some knowledge of surface and colloid chemistry can be a valuable aid to the understanding of a range of biological problems. To date, however, there have been no serious attempts to present in a systematic manner those aspects of surface chemistry which are particularly relevant to biology. Dr Jones has undertaken this quite difficult task and, though he has not produced a definitive work on the subject, he has certainly produced a very readable little volume which should be useful to everyone from undergraduates upwards.

There are eight chapters on topics including surface and interfacial tension, monolayers, micelles, protein-surfactant interactions, electrical double layers, cell surfaces and cell contacts and artificial membranes. In each instance the surface chemical principles are discussed with special regard for biological systems and numerous references are given to the original literature. Herein lies the first of my criticisms, that is, that the references are not, in some cases, as up-to-date as they might be. It may be that this is difficult to avoid in a book on a relatively fast-moving field but, even so, this point should be appreciated. My second concern is that in one or two instances I should like to have seen a more critical presentation of the material. For example, it is not reassuring to find the author putting in a good word for the Pauling-Miller anaesthesia hypothesis, or to see Clements' theory of alveolar stability presented as established fact, or to find that the evidence on the water permeability of bilayers has been inadequately summed up. On most topics, however, the discussion and assessment of the present situation are refreshingly good and, for those starting out in the field, this is a book well worth reading. **D. A. Haydon**

## The Flavonoids

Edited by J. B. HARBORNE, T. J. MABRY and HELGA MABRY  
July 1975: 1162 pages: 412 11960 9: hardback: £27.50

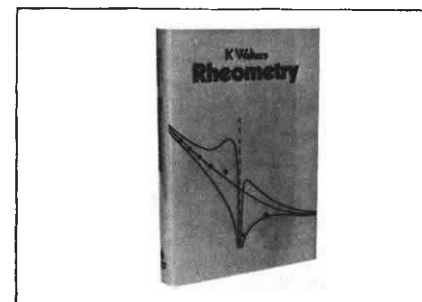


This book provides a comprehensive and definitive account of the flavonoids, one of the most important groups of plant substances. The text covers their recent chemistry, biochemistry, natural distribution and biological importance. The first few chapters deal with techniques of isolation and spectral measurements and with chemical synthesis. There then follow individual chapters covering the fifteen known classes of flavonoid. A significant feature of these chapters is the comprehensive and up-to-date tables listing all known structures within each class of compound. The remaining chapters deal with the enzymology, biosynthesis, metabolism, physiology, systematic distribution, evolution and function of the flavonoids.

## Rheometry

K. WALTERS

July 1975: 288 pages: illustrated: 412 12090 9: hardback: £10.00



There are now many commercially available rheometers with varying degrees of sophistication. The present book is intended to be a text book for such instruments as well as providing a background to rheometry in general.

The development in the book is based on the proposition that there are two basic objectives in rheometry. The first involves a straightforward attempt to characterize the behaviour of non-Newtonian liquids in a number of simple (rheometrical) flow situations, with a view to correlating material behaviour with either molecular structure or observed behaviour in practical situations. The second objective concerns the construction of rheological equations of state for the liquids which can be later used in the solution of flow problems of practical importance.

The author is a mathematician but a genuine attempt has been made to avoid unnecessary mathematical rigour.

**Chapman & Hall**

11 New Fetter Lane, London EC4P 4EE





# announcements

## Appointment

**F. R. Jevons** has been appointed the first vice-chancellor of Deakin University at Geelong, Victoria, Australia.

## Award

**A. M. Weinberg** has been awarded the first **Heinrich Hertz Prize** for contributions to the science and technology of nuclear energy.

## Miscellaneous

**Johann-Georg-Zimmermann Prize.** Intended to encourage cancer research, the prize (50,000 DM) will be awarded to one or more scientists who have earned special recognition in this field. Part of the prize will be awarded in acknowledgement of outstanding work by young scientists (under 40). For this section of the prize, the subject for 1976 will be 'Surgical and radiological treatment of cancer'. Closing date: January 15, 1976. Information: Freunde der Medizinischen Hochschule Hannover e.V., 3000 Hannover 1, Am Hofen Ufer 6, FRG.

## International meetings

September 8–12, **Rhythmic functions in biological systems**, Vienna, Austria (Dr G. Lassmann, c/o Wiener Medizinische Akademie, Alser Strasse 4, 1090 Vienna, Austria).

September 8–12, **Mathematical models for environmental problems**, Southampton, UK (Dr C. A. Brebbia, University of Southampton SO9 5NH, UK).

September 8–12, **Europe from crust to core**, Reading (Mrs D. M. Powell, Local Organising Secretary of MEGS, Department of Geology, The University, Whiteknights, Reading RG6 2AB, UK).

September 8–12, **Applications of ion beams to materials**, Warwick, UK (Professor G. Carter, Department of Electrical Engineering, University of Salford, Salford M5 4WT, Lancashire, UK).

September 8–13, **Carboniferous stratigraphy and geology**, Moscow (Secretary General, Dr Sci Sergei, Viktorovich Meyen, Geological Institute of the USSR Academy of Sciences Carboniferous Organising Committee, Pyzhevsky per 7, Moscow 109017, USSR).

## Person to Person

**Large white butterflies.** References wanted on *Pieris brassicae* L. for inclusion in computerised bibliography covering all aspects of biology of species: unpublished data, odd information or comments, personal observations and information about obscurely published literature (Dr J. Feltwell, Newlyn Cottage, Sutton Valence, Kent, UK).

**Solar neutrinos.** Astrophysicist compiling bibliography, with particular reference to attempted resolutions of the low count rate, would be grateful to receive any information concerning published and unpublished work and/or reprints in this area (A. L. Smith-Haenni, Alpenstrasse 51, 3072 Ostermundigen, Switzerland).

**Edinburgh accommodation.** Wanted for two years, two bedroom flat close to West End if possible (Dr Vagn Mejdahl, Senior Research Fellow, Research Laboratory of National Museum of Antiquities of Scotland, 5/6 Randolph Crescent, Edinburgh EH3 7TE, UK; tel. no. 031-556 8921, ext. 65).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

September 9–10, **Proton transfer**, Stirling, UK (Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, Piccadilly, London W1V 0LQ, UK).

September 9–10, **Morphology and biology of living and fossil reptiles**, London (The Linnean Society of London, Burlington House, Piccadilly, London W1V 0LQ, UK).

September 9–12, **Energy and physics**, Bucharest, Rumania (European Physical Society, PO Box 39, 1213 Petilancy 2, Switzerland).

September 9–12, **Pulsed high beta plasmas**, Abingdon (Mr J. H. C. Maple, Conference Secretary, UKAEA, Culham Laboratory, Abingdon, Oxfordshire ON4 3DB, UK).

September 9–12, **Calcium in biological systems**, Egham, UK (Professor C. J. Duncan, Department of Zoology, The University, PO Box 147, Liverpool L69 3BX, UK).

September 9–13, **Neurosciences**, Munich (Professor D. Ploog, Max-Planck-Institut für Psychiatric, Kraepelinstrasse 10, München 40, FRG).

September 10–12, **Reproductive physiology of invertebrates**, Kerala, India (Dr K. G. Adiyodi, Department of Zoology, Calicut University, Kerala 673635, India).

September 10–12, **Thin-layer chromatography and associated techniques**, London, UK (Laboratory News Europe, 78 Wigmore Street, London W1, UK).

September 10–17, **Virology**, Madrid (Dr. R. Najera, Centro Nacional de Virologia y Ecologia, Sanitarias, Majadahonda, Madrid, Spain).

September 11–12, **Computers in laboratory use**, London (The Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

September 11–18, **Neurology**, Amsterdam (Preliminary Congress Secretariat, University Clinic of Neurology, Paviljoen 2, Wilhelmina Gasthuis, Amsterdam, The Netherlands).

September 12–21, **Surface membrane receptors**, Bellagio, Italy (Dr R. A. Bradshaw, Director, Department of Biological Chemistry, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, Missouri 63110).

September 13–17, **Transplutonium**, Baden-Baden, German Federal Republic (Professor W. Muller, European Institute of Transuranium Elements, Kernforschungszentrum Karlsruhe, Postfach 22600, 7500 Karlsruhe, German Federal Republic).

September 14–19, **Environmental monitoring**, Las Vegas, Nevada (E. A. Schuck, National Environmental Research Center, US Environmental Protection Agency, PO Box 15027, Las Vegas, Nevada 89114).

September 15–16, **Prostaglandin**, Halle/Saale, DDR (Professor W. Forster, Institut für Pharmakologie und Toxikologie der Universität, 402 Halle/Saale, Leninallee 4, DDR).

September 15–19, **Beam-foil spectroscopy**, Gatlinburg (Professor W. W. Havens, Jr, American Physical Society, 335 East 45th Street, New York, New York 10017).

September 15–19, **Liquid scintillation counting**, Bath, UK (Mr M. A. Crook, The Society for Analytical Chemistry, 9–10 Savile Row, London W1X 1AF, UK).

September 15–20, **Cnidarian fossils**, Paris (Dr J. P. Chevalier, Institut de Paleontologie, 8 rue de Buffon, 75005 Paris, France).

September 16–17, **Fluidised combustion**, London (Dr P. Eisenklam, Department of Chemical Engineering, Imperial College, London SW7 2AZ, UK).

September 16–18, **Neurohypophysial hormones**, Liblice, Czechoslovakia (Dr M. Zaoral, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha 6, Felmingovonam 2, Czechoslovakia).

September 16–19, **Optical fibre communications**, London (The Conference Department, The Institution of Electrical Engineers, Savoy Place, London WC2R 0BL, UK).

September 16–19, **Offshore Europe**, Aberdeen (Exhibition Administration Limited, 4 Pine Walk, Surbiton, Surrey, UK).

September 17–19, **Growth hormone and related peptides**, Siena, Italy (Mrs M. L. Pecile, Department of Pharmacology, School of Medicine, University of Milano (SM-UM), 32 Via Vanvitelli, 120129 Milano, Italy).

September 17–19, **Clinical biochemistry**, Bratislava (R. Muller, Congress Office, Slovak Medical Society, Mickiewiczova 18, 88322 Bratislava, Czechoslovakia).

September 17–20, **Kinetics and biopolymers**, Jena, DDR (Professor H. Berg, Zentralinstitut für Mikrobiologie und Experimentelle Therapie der AdW, 69 Jena, Beutenbergstrasse 11, DDR).

September 21–24, **Electromyography**, Rochester, Minnesota (W. C. Wiederholt, Secretary Treasurer, 7010 Via Valverde, La Jolla, California 92037).

September 21–25, **Immunology of reproduction**, Varna, Bulgaria (Third Symposium on Immunology of Reproduction, 73 Lenin Avenue, Sofia 13, Bulgaria).

## Reports and publications

### Great Britain

Wildlife Conservation in Charnwood Forest—Report by a Working Party. Pp. 51. (Huntingdon: East Midlands Regional Office, Nature Conservancy Council, Monks Wood Experimental Station, Abbots Ripton, 1975.) £1.50 [205]

Proceedings of the Conference on Animal Feeds of Tropical and Subtropical Origin, held at the London School of Pharmacy, Brunswick Square, London, WC1N 1AX, 1st–5th April 1974. Pp. 347. (London: Tropical Products Institute, 56/62 Grays Inn Road, WC1, 1975.) £4.05. [205]

Flora of Tropical East Africa. Edited by R. M. Polhill. Melastomataceae. By Dr. G. E. Wickens. Pp. 95. £1. Dioscoreaceae. By E. Milne-Redhead. Pp. 26. 35p. (London: Crown Agents for Overseas Governments and Administrations, 1975.) [215]

Bulletin of the British Museum (Natural History). Geology. Vol. 25, No. 5: A Revision of Sahni's Types of the Brachiopod Subfamily Carneithyridinae. By U. Asgaard. Pp. 317–365 + 8 plates. £4.50. Zoology. Vol. 28, No. 1: A Guide to the Species of the Genus *Euplotes* (Hypotrichida, Ciliata). By C. R. Curds. Pp. 1–61. £3.80. Vol. 28, No. 2: Catalogue of the Types of Terrestrial Isopods (Oniscoidae) in the Collections of the British Museum (Natural History). II. Oniscoidae, Excluding Pseudotracheata. By J. P. Ellis and R. J. Lincoln. Pp. 63–100. £2.55. Vol. 28, No. 3: The Larval Development of *Carcinus maenas* (L.) and *Mediterraneanus Czerniavsky* (Crustacea, Brachyura Portunidae) Reared in the Laboratory. By A. L. Rice and R. W. Ingle. Pp. 101–119 + 1 plate. £1.35. Vol. 28, No. 4: A Comparative Study of the Larval Morphology of the British Portunid Crabs *Macropipus puber* (L.) and *M. volatus* (Fabricius), with a Discussion of Generic and Sub-Familial Larval Characters within the Portunidae. By A. L. Rice and R. W. Ingle. Pp. 121–151. £1.90. (London: British Museum (Natural History), 1975.) [225]

Department of Industry. Technology and the Environment. (Reports from Scientific Counsellors Overseas, No. 8.) Pp. 34. (London: Department of Industry, Abel House, John Islip Street, SW1, 1975.) [225]

Cotton Research Corporation. Annual Report for 1974. Pp. 19. (London: Cotton Research Corporation, 1975.) [275]

Proceedings of the Royal Irish Academy. Vol. 75, Section A, No. 9: Thermodynamic Influences on the Propagation of Electromagnetic Shock Waves. By M. J. McCarthy and P. M. O'Leary. Pp. 85–96. 40p. Vol. 75, Section B, No. 12: Studies on the Synthesis of Disaccharides—I. Synthesis of Gentioibiose and 6,0-β-D-Galactopyranosyl-D-Galactose. By Elizabeth E. Lee, Angela Hartigan and P. S. O'Colla. Pp. 267–273. 17p. No. 13: Irish Botany in the Seventeenth Century. By M. E. Mitchell. Pp. 275–284. 26p. No. 14: The Food of the Cormorant *Phalacrocorax carbo* at Some Breeding Colonies in Ireland. By B. West, D. Cabot, and M. Greer-Walker. Pp. 285–304. 50p. (Dublin: Royal Irish Academy, 1975.) [275]

List of University Institutions in the Commonwealth. Pp. 32. (London: The Association of Commonwealth Universities, 1975.) [275]

University of Cambridge. Report of the Head of the Department of Engineering for the Academic Year 1973–74. Pp. 10. (Cambridge: The University, 1975.) [285]

### Other countries

Beiträge zur Geobotanischen Landesaufnahme Der Schweiz. Heft 55. Vegetationsentwicklung und Waldgrenzschwankungen des Spät- und Postglazials im Oberhalbstein (Graubünden/Schweiz) mit besonderer Berücksichtigung der Fichteneinwanderung. Von Christian Heitz. Pp. 63. (4 Diagrams.) (Bern: Verlag Hans Huber, 1975.) Sw. Fr. 30. [75]

Doctors and Healers. By Alexander Dorozynski. Pp. 63. (Ottawa: International Development Research Centre, 1975.) [85]

Republic of the Sudan. Ministry of Agriculture, Agricultural Research Division. 1967–1968 Annual Report of the Hudeiba Research Station. Pp. 78. (Wadi Medani: Agricultural Research Corporation.) n.p. [95]

Geophysical Institute, University of Alaska. Annual Report, 1973/1974. Pp. 169. (Fairbanks, Alaska: Geophysical Institute, University of Alaska, 1975.) [125]

Methodological Guidelines for Social Assessment of Technology. Pp. 146. (Paris: OECD; London: HMSO, 1975.) 22 francs; £2.20; \$5.50. [125]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 228: Quaternary Geology and Geomorphology of Assiniboine and Qu'appelle Valleys of Manitoba and Saskatchewan. By R. W. Klassen. Pp. 61. \$4. Bulletin 243: The Jurassic Faunas of the Canadian Arctic. By Hans Fredöhl. Pp. 34 (5 plates). \$4. Paper 74-16: Jurassic and Lower Cretaceous Paleogeography and Depositional Tectonics of Porcupine Plateau, Adjacent Areas of Northern Yukon and Those of Mackenzie District. By J. A. Jelezky. Pp. 52. \$2.50. Paper 74-63: Computer-Based Systems for Geological Field Data. (An International State-of-the-Art Review for 1973 conducted by COGEO DATA, International Union of Geological Sciences, in collaboration with the Division of Earth and Environmental Sciences, UNESCO, and the Canadian Centre for Geoscience Data.) By W. W. Hutchison. Pp. 100. (Ottawa: Information Canada, 1975.) \$4.80. [155]

Bulletin of the Fisheries Research Board of Canada. No. 190: Hydrodynamics and Energetics of Fish Propulsion. By P. W. Webb. Pp. x + 158. \$6. No. 192: Catalogue and Synopsis of *Caligus*, a Genus of Copepoda (Crustacea) Parasitic on Fishes. By L. Largoli, Z. Kabata and R. R. Parker. Pp. vi + 117. \$6. (Ottawa: Information Canada, 1975.) [155]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1999-N: Quality of the Ground Water in Basalt of the Columbia River Group, Washington, Oregon, and Idaho. By R. C. Newcomb. Pp. iv + 71. (Washington, DC: Government Printing Office, 1972.) 75 cents. [195]

Smithsonian Contributions to Zoology. No. 178: Revision of the Cyprinidae of the Gulf of Naples (Ostracoda). By Louis S. Kornicker. Pp. 64. \$1.95. No. 179: West African Myodocopid Ostracoda (Cylindroleberitidae). By Louis S. Kornicker and Francisca Elena Carrión. Pp. iii + 78. \$1.75. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [195]

The Right to Conduct Nuclear Explosions: Political Aspects and Policy Proposals. (Stockholm Paper No. 6.) Pp. 24. (Stockholm: Stockholm International Peace Research Institute, 1975.) [195]

Chemical Disarmament: New Weapons for Old (Stockholm International Peace Research Institute Monograph) Pp. viii + 151. (Stockholm: SIPRI and Almqvist and Wiksell International; New York: Humanities Press, 1975.) [195]

Safeguards Against Nuclear Proliferation. (A SIPRI Monograph.) Pp. viii + 114. (Stockholm: Almqvist and Wiksell International; Cambridge, Mass. and London: The MIT Press, 1975.) Sw. kr. 49. [195]

CERN—European Organization for Nuclear Research. CERN 75–3: Radiation and Fire Resistance of Cable-Insulating Materials Used in Accelerator Engineering. By H. Schinbacher and M. H. Van de Voorde. Pp. 15. (Geneva: CERN, 1975.) [195]

Annalen der Meteorologie (Neue Folge). Nr. 9, Die Meteorologen-Tagung in Bad Homburg v.d.H.: vom 27. bis 29. März 1974. Pp. 161. Berichte des Deutschen Wetterdienstes. Nr. 134 (Band 17): Entwicklung eines Sky-Scanners zur Schnellen Vermessung der Räumlichen Verteilung Spektraler Himmelsstrahlungen. Von Klaus Deene. Pp. 37. Nr. 135 (Band 17): Zwei Wetterkatastrophen des Jahres 1972—Der Niedersachsen-Orkan und das Gewitterunwetter von Stuttgart. Von A. Cappel und P. Emmrich. Pp. 84. (Offenbach A.M.: Selbstverlag des Deutschen Wetterdienstes, 1974 und 1975.) [205]

Records of the Australian Museum, Vol. 29, No. 11: Contributions to the Knowledge of the Alpheid Shrimp of the Pacific Ocean. Part XVIII: A New Species of the Genus *Alpheus* from the Mouth of the Sepik River, New Guinea. By Albert H. and Dora M. Vanner. Pp. 261–266. (Sydney: The Australian Museum, 1975.) 50 cents. [205]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1880-B: Floods of September–October 1967 in South Texas and North-eastern Mexico. By Elmer E. Schroeder, R. U. Grozier, D. C. Hahl and A. E. Hulme. Pp. vi + 111. (Washington, DC: Government Printing Office, 1974.) \$3.55. [215]

Smithsonian Contributions to Zoology. No. 184: The Genus *Coptocarpus* Chaudoir of the Australian Region with Notes on Related African Species (Coleoptera: Carabidae: Oodini). By Terry L. Erwin. Pp. 25. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 85 cents. [215]

Psychopharmacology Communications, Vol. 1, No. 1, 1975. Pp. 1–115. Subscription rate for Vol. 1 (1975) containing six issues \$35 (prepaid). Postage outside the United States is \$6.39 per volume. (New York: Marcel Dekker Journals, 1975.) [225]

A National Statement by the Faculties of Agriculture and Veterinary Medicine at Canadian Universities. Pp. 22. (Ottawa: Science Council of Canada, 1975.) [225]

Australian Academy of Science. Science and Industry Forum Report No. 7: PhD Education in Australia—The Making of Professional Scientists. Pp. 212. (Canberra, ACT: Australian Academy of Science, 1974.) [275]

The Epidemiology of Cancer in Papua New Guinea. Pp. 184. (Port Moresby, Papua: Department of Public Health, 1974.) \$10. [275]

Official Records of the World Health Organization No. 221: The Work of WHO 1974. (Annual Report of the Director-General to the World Health Assembly and to the United Nations.) Pp. xviii + 343. (Geneva: WHO: London: HMSO, 1975.) Sw. fr. 18. [275]

Environment Canada. Fisheries and Marine Service. Technical Report No. 521: Eggs, Larvae and Juveniles of Fishes from Plankton Collections in the Gulf of St. Lawrence During 1969. By A. C. Kohler, D. J. Faber and N. J. McFarlane. Pp. 154. No. 534: Underwater Biotelemetry, an Annotated Bibliography. By Aivars B. Stasko. Pp. 29. (St. Andrews, New Brunswick: Research and Development Directorate, Biological Station, 1975.) [285]

Bulletin of the American Museum of Natural History. Vol. 155, Article 2: A Taxonomic Study of African Alloadapine Bees (Hymenoptera, Anthophoridae, Ceratinini). By Charles D. Michener. Pp. 67–240. (New York: American Museum of Natural History, 1975.) \$8.55. [295]

Smithsonian Contributions to Zoology. No. 181: The Costate Species of *Colaspis* in the United States (Coleoptera: Chrysomelidae). By Doris H. Blake. Pp. iii + 24. (Washington, DC: Smithsonian Institution Press 1974. For sale by US Government Printing Office.) 65 cents. [295]

Smithsonian Contributions to Zoology. No. 186: A Revision of the South American Fishes of the Genus *Nannostomus* Günther (Family Lebiastidae). By Stanley H. Weitzman and J. Stanley Cobb. Pp. iii + 36. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) \$1.10. [305]

United States Department of the Interior: Geological Survey. Professional Paper 726-C: Gravity and Magnetic Features as Related to Geology in the Leadville 3-Minute Quadrangle, Colorado. By Ogden Tweto and J. E. Case. Pp. iii + 31 + plate 1. (Washington, DC: Government Printing Office, 1972.) [305]